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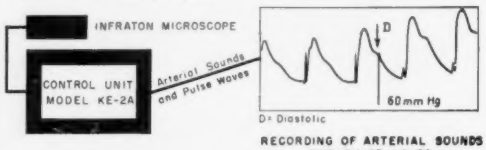

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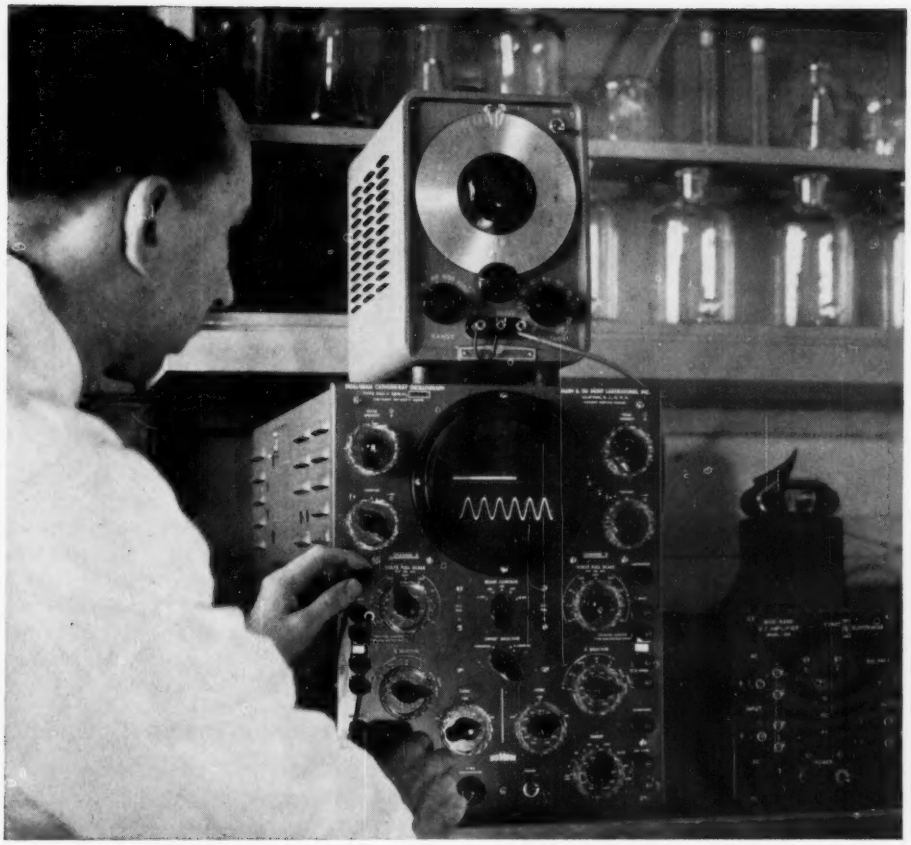

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1. Proc. Soc. Exper. Biol. & Med. *89*:281 (June) 1955. 2. Lorand, L.: Physiol. Rev. *34*:742, 1954. 3. J.A.M.A. *159*:771 (Oct. 22) 1955. 4. J. Clin. Investigation *34*:1520 (Oct.) 1955.

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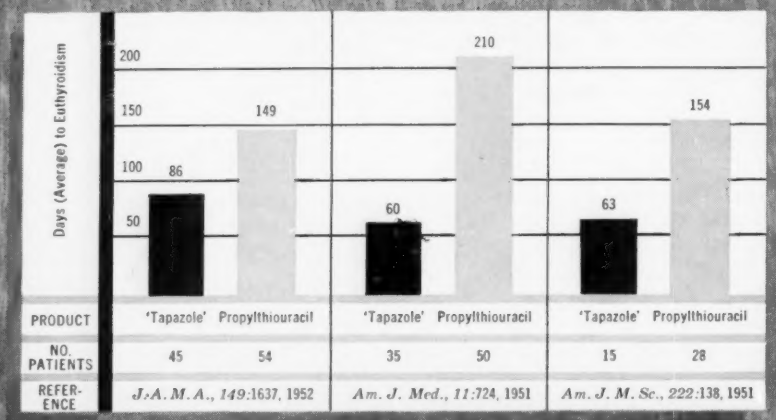

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POSTURAL EFFECTS ON RENAL TUBULAR ACTIVITY[1]

By A. SURTSHIN and H. L. WHITE

(From the Department of Physiology, Washington University School of Medicine, St. Louis, Mo.)

(Submitted for publication October 10, 1955; accepted October 27, 1955)

A number of observers have reported that acute reduction of glomerular filtration rate (GFR) in experimental animals induces a considerable fall in renal excretion of sodium and water. Chronic constriction of one renal artery is followed by persistent fall in salt and water excretion by that kidney (1). In man, change from the recumbent to the erect position is followed by a considerable fall in urinary flow and sodium excretion and by small decrease in inulin and exogenous or endogenous creatinine clearances (2–4). The above findings were on subjects not in maximal water diuresis. In human subjects with a large water load who had been sitting for four to eight hours, change to recumbency was followed by increase in urine flow, sodium excretion and sodium urinary concentration while creatinine U/P ratios fell and endogenous creatinine clearance rose slightly (5).

Whether more nearly complete reabsorption of sodium and water following maneuvers reducing GFR is accomplished in the proximal or the distal segment of the tubule is not certain. Pitts and Duggan (6, 7) inferred that organic mercurial diuretics depress the distal reabsorption of sodium, and after observing that aortic constriction produced marked fall in sodium excretion even after administration of a large dose of Mercuhydrin® in dogs they concluded that the increased sodium and water reabsorption resulting from fall in GFR takes place in the proximal segment. The inference that mercurial diuretics act solely distally has been supported (8) and rejected (9, 10) and the loci of the more complete reabsorption remain unidentified.

As has been reviewed previously (4) most workers have found a fall in GFR with postural change from the horizontal to a head-up tilt or standing. We here report the effects of postural change on the excretion of water, sodium, creatinine and osmotically active solutes in normal human subjects *in maximum water diuresis.* We have attempted to localize within the nephron some of the changes observed, using the following assumptions: 1) Distal water reabsorption is abolished in maximum water diuresis with the subject at ease, recumbent or standing (11). 2) Proximal reabsorption is isotonic (12–15). Evidence for the first assumption is that urine flow in maximal water diuresis or in complete diabetes insipidus about equals the volume of fluid leaving the proximal tubules, as estimated by observations on proximal fluid samples obtained by micropuncture (13).

If it should be found that the percentage fall in water excretion on changing from recumbency to standing with subjects in maximal water diuresis is significantly greater than the percentage fall in GFR it would follow that the percentage of filtered water reabsorbed proximally is increased on standing. Since proximal reabsorption is considered as isotonic, the percentage of filtered sodium reabsorbed proximally is also increased. If it should further be found that urine and plasma osmolarity remain unchanged, free water clearance also shows a greater percentage fall than does GFR. This means that distal reabsorption of osmotically active solutes, largely sodium salts, without water shows a greater percentage fall than does GFR, and since distal reabsorption of water is taken as zero, distal reabsorption of solute leading to free water is the same as total distal reabsorption of solute. We would thus conclude that on changing from recumbency to standing in maximum water diuresis the percentage of filtered water and sodium reabsorbed proximally is increased, while the percentage (and absolute amount per unit time) of sodium reabsorbed distally is decreased. The same conclusions would follow from any combination of circumstances leading, in maximal water diuresis, to a greater percentage fall in urine flow and free water clearance than in GFR. Although we have used sub-

[1] Aided by grant A-92 (C4), National Institutes of Health, USPHS.

jects in maximal water diuresis in order to equate volume delivered to the distal segment with urine flow (assumption 1), we have no reason to believe that the postural responses of the proximal segment in the reabsorption of water or sodium, or of the distal segment in the reabsorption of sodium, would be any different at lower urine flows.

METHODS

Subjects reported to the laboratory between 8:00 and 9:30 A.M. after having had a light breakfast without added salt, and water *ad libitum*, but no other food since about the previous 7:00 P.M. They drank 1,200 cc. of tap water in 10 or 15 minutes, emptied their bladders and then either lay down on a cot or remained standing. Every 20 minutes thereafter for 1 hour (3 doses) they drank 300 cc. of tap water. The bladder was emptied at the end of the first hour, urine volume was recorded and the positive water load (2,100 cc. minus urine volume) was maintained constant thereafter, neglecting extrarenal water loss. After the first hour the bladder was emptied every 15 minutes and an amount of tap water equal to the volume of urine passed was drunk. The procedure was continued until urine flow was reasonably constant for four or more periods when a venous blood sample was taken. After one or two additional periods (during which urine flow did not diminish) change in posture from supine to standing or vice versa was effected with voiding and drinking continued as before. The new posture was maintained until five or more consecutive periods with constant urine flow were obtained. Another venous blood sample was then taken

TABLE I

Postural effects on urine flow and solute excretion *

Subject, Date	Posture change	Water load cc.	Creat. clear. cc./min.	Urine flow cc./min.	Urine Na mEq./L.	Urine Na μEq./min.	Urine solutes mOs./L.	Urine solutes mOs./min.	Plasma solutes mOs./L.	Osmol. clear. cc./min.	Free water cc./min.	Creat. U/P
H W												
3/16	L–S†	1,700	100	8.46	10.6	85.7	70.5	.598	275.0	2.17	6.29	11.8
			108	15.4	17.2	265	74.5	1.15	275.0	4.19	11.2	7.2
			.92	.55	.62	.32	.95	.52		.52	.56	1.64
3/25	S–L	1,860	75.9	7.15	3.22	23.1	64.5	.477		1.73	5.42	10.7
			89.9	11.2	11.6	130	67.0	.721	276.0	2.61	8.59	8.1
			.84	.64	.28	.18	.96	.66		.66	.63	1.32
W S												
3/18	L–S	1,920	145	12.5	7.16	90.0	63.5	.797	278.5	2.86	9.64	11.6
			145	14.4	8.23	119	69.5	1.01	292.0	3.44	11.0	10.0
			1.00	.87	.87	.76	.91	.79		.83	.88	1.16
6/27	S–L	1,878	117	16.6	4.81	79.8	53.5	.886	282.0	3.14	13.5	7.2
			122	18.7	7.40	138	49.0	.915	286.5	3.19	15.5	6.5
			.96	.89	.65	.58	1.09	.97		.97	.87	1.11
M K												
6/21	L–S	1,895	115	10.3	14.0	145	73.0	.753	275.0	2.73	7.57	11.4
			116	15.3	16.2	247	80.0	1.22	276.5	4.41	10.9	7.4
			.99	.67	.86	.59	.91	.62		.62	.69	1.54
7/18	L–S	1,670	130	12.8	14.2	181	66.0	.842	272.0	3.11	9.69	10.2
			133	17.0	19.3	333	75.5	1.28	270.5	4.74	12.3	7.8
			.98	.75	.74	.54	.87	.66		.66	.79	1.31
A S												
8/18	L–S	1,897	85.0	12.2	9.86	120	60.0	.731	274.0	2.65	9.55	7.0
			89.5	15.6	10.4	162	61.5	.954	277.5	3.46	12.1	5.8
			.95	.78	.95	.74	.98	.77		.77	.79	1.21
	Average		94.9	73.6	71.0	53.0	95.3	71.3		71.9	74.4	1.33

* Averages of values for five periods at the plateaus. Standing values in the numerator and recumbent values in the denominator with the decimal equivalents below.

† S = Standing.
L = Lying.

and the experiment terminated. Experiments were conducted with the subjects quiet, comfortable and undisturbed in a room regulated at about 27° C. Smoking was not permitted. Voluntary voiding was accomplished without change of posture and without significant retention as evidenced by the creatinine excretion data. While standing the subject occasionally shifted his feet or walked a few steps so as to prevent excessive venous pooling in the lower extremities. Seven experiments in four apparently healthy male subjects were done. In the experiment on MK on 7/18 the procedure was varied only in that between noon and 10:00 P.M. of the previous day the subject ingested 200 mEq. of sodium chloride in isotonic solution in divided doses. The measurements recorded were period length, urine volume, urine and plasma osmolarity (Fiske Osmometer), urine sodium concentration (Weichselbaum-Varney Universal Spectrophotometer) and plasma and urine creatinine concentrations (16).

RESULTS

The data used hereafter (Table I) are the averages of the values for the last five periods in each position. In all subjects the plateau of urine flow during water diuresis with a large constant positive water load was lower during standing than during recumbency. In one subject (WS) it was as high as 89 per cent and in the other 3 was from 55 to 78 per cent of that in recumbency. Endogenous creatinine clearance during standing varied from 84 to 100 per cent of the value for lying.

During standing urine sodium concentrations were 28 to 95 per cent and urine sodium excretion rate 18 to 76 per cent of the values for recumbency. Plasma creatinine and osmolar concentrations varied little during the course of individual experiments. In 6 experiments standing osmolar clearance ranged from 52 to 77 per cent of the value while lying, while in one experiment in which urine osmolarity was slightly lower during recumbency (after change from standing to lying), standing osmolar clearance was as much as 97 per cent of the value for lying.

Free water clearance in the erect position was always less than during lying since the absolute fall in urine flow was greater than the absolute fall in osmolar clearance. In all instances creatinine U/P was higher during standing. In the two experiments in which the plasma protein concentration was determined (17) in the venous blood samples (HW 3/16 and WS 3/18) the values rose 4 and 6 per cent on standing.

DISCUSSION

To permit the assumption that distal water reabsorption was absent during these experiments maximal water diuresis was employed. The validity of any inferences as to proximal versus distal sites of reabsorption depends on the validity of the two assumptions stated earlier. Change in posture from lying to standing or the reverse was used to induce changes in GFR. In a previous communication (4) the literature has been summarized to indicate that a) most workers find a fall in filtration rate on changing from the horizontal to a head-up tilt or to standing, b) all workers find falls in water and sodium excretion on changing from the horizontal to a head-up tilt or to standing. Additional relevant reports have since been published (5, 18–20).

Under the conditions of high water loading employed with resultant maximal rates of urine flow endogenous creatinine clearance (used as an index of GFR) was not diminished as much during standing as is usually seen with lower rates of flow. While changes in the creatinine clearance in 5 of the 7 experiments were within the limits of experimental error, values during standing never exceeded those during recumbency. We believe that GFR was really lower to a small and variable extent during standing than lying. The situation is much the same as is seen with mild unilateral renal artery constriction in the dog (1). There, as in the present experiments, fall in excretion of water and sodium was clearly evident in the presence of GFR values which in any single experiment could not with certainty be called depressed, but which were uniformly slightly lower than the control.

In each of our experiments the percentage by which urine flow is lower during standing than lying exceeds that for endogenous creatinine clearance. Under our assumptions we conclude therefore that with small lowering of GFR proximal reabsorption of sodium and water becomes more nearly complete.

Our finding is that free water clearance may be somewhat different on different occasions in the same individual in maximal water diuresis. With greater rates of urine flow greater levels of free water are achieved since the absolute differences in osmolar clearance on different occasions are

small relative to those in urine flow. The free water values of the various periods of a plateau of a given standing or lying session did not vary among themselves by more than about 20 per cent. Since we used no solute loading in the course of an experiment we have no data on whether free water clearance at flows above those obtained by maximal sustained simple water diuresis remains constant (9, 21) or not (5). Our values were usually in the range described by Smith (21). In subject MK greater urine flow and free water clearance were obtained after light salt loading than without loading.

Since the percentage by which free water was decreased during standing was about the same as that for decrease in urine flow it follows that distal reabsorption of sodium remains about the same percentage of the distally presented load in the two positions, but the percentage of the filtered sodium reabsorbed distally falls on standing. No information is derived as to the existence of a maximal distal tubular capacity for sodium reabsorption since the sodium load presented distally on standing approached or fell below the osmolar equivalent of the free water clearance obtaining during lying in the same experiment.

We assume that in the presence of water loading pituitary antidiuretic hormone action on the kidney is absent in both the erect and recumbent positions. Although this may be open to some question, our subjects did not have the circulatory disturbances present in the subjects of Brun, Knudsen, and Raaschou (22, 23). In the experiments of Pearce and Newman (19) in which water diuresis was not maximal, a head-up tilt usually produced a greater percentage fall in water excretion than in electrolyte excretion with resultant increase in electrolyte concentration. In our experiments sodium concentration was always considerably lower and osmolar concentration usually slightly lower in the erect position than in the horizontal. Rise in creatinine, inulin or mannitol U/P cannot be taken as proof of ADH action since it may be seen on constriction of the aorta or of a renal artery (1, 24, 25) where there is no reason to implicate ADH. A rise in mannitol U/P also occurs in normal or uncontrolled diabetes inspidus subjects with abdominal compression (26), although here the possibility exists that the discomfort attending the procedure may have elicited ADH secretion even in clinical diabetes insipidus subjects.

While the possibility of a distal $T^c_{H_2O}$ process (reabsorption of water without solute) operating continuously (even in maximum water diuresis) at a level of 5 to 7 cc. per minute (27) cannot be denied, its probability is low. On the basis of a constant $T^c_{H_2O}$ of 6 cc. per minute, calculations still show that a higher percentage of the filtered water and sodium is reabsorbed proximally on standing than on lying, and that a lower percentage of the filtered sodium is reabsorbed distally on standing, although the calculated changes are less than on the basis of our first assumption.

SUMMARY

The effects of posture on urine flow, free water clearance, osmolar clearance, endogenous creatinine clearance and sodium excretion were determined in normal subjects in maximal water diuresis. It is assumed that distal water reabsorption is abolished and that proximal reabsorption is isosmotic. The findings are interpreted to mean that a higher percentage of the filtered water and sodium is reabsorbed proximally on standing than on lying and that a lower percentage of the filtered sodium (and a lower absolute amount per unit time) is reabsorbed distally on standing. The percentage reabsorbed of the distally delivered sodium load is the same for lying and standing. Slightly lower endogenous creatinine clearance values were observed on standing, although the differences between standing and lying were less than are seen at urine flows below maximum. The percentage changes in creatinine clearance were less than in excretion of water and sodium and in free water and osmolar clearances.

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THE HEMODYNAMICS OF THYROTOXICOSIS IN MAN WITH SPECIAL REFERENCE TO CORONARY BLOOD FLOW AND MYOCARDIAL OXYGEN METABOLISM[1]

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For many years the hemodynamics of thyrotoxicosis have been of interest to clinicians. Blood flow through the extremities was assumed to be increased in thyrotoxicosis because of the high rate of heat elimination when the hand was placed in a calorimeter (1). Several workers found an increase in cardiac output in thyrotoxic subjects using the oxygen content of arm vein blood in the Fick formula (2–4). Similar findings were obtained using the nitrous oxide (5) and acetylene methods (6, 7). The velocity of circulation, as determined by the rate of transit of radioactive materials through the cardiovascular system is accelerated in thyrotoxicosis and becomes normal after effective treatment (8, 9). Circulation time measured by sodium dehydrocholate in patients with thyrotoxicosis is more rapid than normal (10).

Blalock and Harrison using oxygen content of right ventricular blood in the Fick formula showed that in dogs thyroid feeding produced an increase in cardiac output while thyroidectomy produced a decrease in output (11).

Experiments concerning the myocardium directly have shown an increase in O_2 uptake/gram of muscle. Direct measurement of O_2 consumption in the Warburg apparatus demonstrated an increase in oxygen utilization by the thyroid stimulated myocardium as shown in the isolated beating rabbit auricle (12). Dock and Lewis established that thyroid feeding to rats increased the total body oxygen consumption (13). Then by converting the animals to a heart lung preparation they demonstrated that the increased oxygen consumption by the heart and lungs was directly related to the cardiac rate and the oxygen consumption per beat remained unchanged. They concluded that the increase in cardiac oxygen metabolism was explained by the increased cardiac rate and was not otherwise related to the hypermetabolic state.

In view of this rather consistent body of data the report of normal myocardial oxygen consumption in thyrotoxic patients despite an increase in total metabolism is surprising (14). It appeared that these observations might be re-evaluated in a larger number of patients, studying them before and after treatment for thyrotoxicosis so that each patient could serve as his own control.

MATERIAL AND METHODS

Hemodynamic studies were made of ten patients believed to have thyrotoxicosis on the basis of clinical and laboratory examination. The plan was to determine the cardiac output and coronary blood flow of each patient in the thyrotoxic state, to treat him with radioactive iodine or surgery, and subsequent to clinical improvement to repeat the hemodynamic studies. All patients were treated with radioactive iodine except No. 6 (L. M.), who had subtotal thyroidectomy.

All studies were done in the fasting, post-absorptive state and every effort was made to obtain basal conditions. Cardiac output was determined by the Fick principle, coronary blood flow by the nitrous oxide saturation method utilizing a blood-myocardial partition coefficient of 1 (15). Pressures were recorded with Statham strain gauges and the direct writing Sanborn poly-viso. The usual formulae were used for calculation of cardiac work and peripheral resistance.

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The final series consists of eight acceptable complete studies. The ninth patient, (F. K.) had the lowest initial I^{131} uptake in the series, 49 per cent, and although the radioactive iodine uptake decreased following therapy he did not attain a satisfactory clinical response. He failed to gain weight and continued to experience his symptoms of nervousness, tachycardia, palpitation and dyspnea. Additional investigation at this time revealed a severe anxiety tension state associated with a very difficult home situation. This patient's data are shown at the bottom of the table but are excluded from the averages because it is felt that his primary disease was not thyrotoxicosis. However, statistical calculations were made with these data included and excluded (Table I) without producing significant differences in the over-all pattern. The tenth patient was a 22-year-old woman with moderately severe thyrotoxicosis and mild hypertension who returned to the hospital in the euthyroid state subsequent to treatment. However, because she was pregnant and had developed more severe hypertension, it was felt that data collected from her would serve no purpose for this series so re-study was not done. In patient G. A. a satisfactory initial determination of coronary blood flow was impossible from the first nitrous oxide curves but since he was still thyrotoxic at the time of his return to the hospital hemodynamic studies were repeated before and after he had responded to further treatment. Results in this case will be cited later. Comparisons were also made between hyperthyroid, treated hyperthyroid and nine normal subjects studied in our laboratory.

RESULTS

Results of the study are summarized in Table I. It is apparent that these patients responded to treatment of thyrotoxicosis in the usual fashion with a gain of weight, decrease in heart rate and oxygen consumption per square meter body surface and a lessening of radioactive iodine uptake by the thyroid. Although no significant change occurred in the systemic mean arterial blood pressure, there was a fall in the pulmonary arterial blood pressure ($p < 0.01$). This differing response in the two circulations resulted in a significant increase in total peripheral resistance ($p < 0.05$) with no change in the pulmonary resistance. There was a 50 per cent decrease in right ventricular work index ($p < 0.01$) but, although the left ventricular work index decreased 28 per cent, this was not significant ($p < 0.1$). However, the major change in cardiac output in patient G. A. occurred after his initial, but only partially successful, treatment. Although this study was incomplete, the cardiac output data are satisfactory. If the second study be replaced by this determination, statistical analysis reveals a significant decrease in left ventricular work index for the entire group ($p < 0.05$). The decrease in rate (-28 per cent) was so similar to the fall in cardiac index (37 per cent) that no significant change in stroke index occurred.

Observations on the myocardial metabolism revealed that successful treatment was followed by significant changes in the coronary vascular resistance ($+70$ per cent $p < 0.01$), coronary blood flow (-38 per cent $p < 0.01$), cardiac metabolic rate for oxygen per 100 grams of myocardial tissue (-37 per cent $p < 0.01$) and the cardiac metabolic rate for oxygen per beat (-17 per cent $p < 0.05$). When the patients with thyrotoxicosis were compared to normal subjects, see Table I, there were significant increases in heart rate ($p < 0.01$), oxygen consumption ($p < 0.02$), mean pulmonary arterial pressure ($p < 0.05$), cardiac index ($p < 0.05$), left and right ventricular work index ($p < 0.02$), coronary blood flow ($p < 0.05$), and cardiac oxygen utilization ($p < 0.05$) in the former. Our data concerning the normal coronary blood flow and myocardial oxygen extraction and utilization compare favorably with Bing's 18 normal subjects (14). Conversely, following treatment, comparison of post-thyrotoxic patients with normals revealed no significant differences in the hemodynamic and metabolic state.

DISCUSSION

This study indicates that contrary to previously published observations (14) the myocardium participates in the increase in oxygen consumption characteristic of all body tissues in thyrotoxicosis. This hypermetabolism is accompanied by an increase in coronary blood flow, a decrease in coronary vascular resistance, and an increase in oxygen consumption per beat. It seems clear since there is an increase in oxygen consumption per beat that the cardiac oxygen utilization is related not only to the increase in cardiac rate but to the general hypermetabolism of the myocardium as well. If the left ventricular work or left ventricular work index be divided by the myocardial oxygen consumption per 100 grams of tissue, an index of efficiency is obtained. Although these indices did not change, comparison of figures obtained by this method would be subject to errors inherent in

TABLE I

*Hemodynamics in thyrotoxicosis before and after treatment**

Patient	Date	Age	Sex	Wt. *Kg.*	S.A. M^2	I^{131} uptake %	BMR %	Heart rate	Syst. MABP	P.A. MABP	O_2 cons. *cc./M²*	Art. O_2 *Vol. %*	M. ven. O_2 *Vol. %*	Δ A-V O_2 *Vol. %*	Cardiac index	L.V. work index
1. W. H.	5/12/54	11	M	34	1.22	63	+50	120	92	22	241	16.7	13.3	3.4	7.2	8.9
No. 288722	8/11/54			39	1.29	11	+18	66	104	8	132	18.4	13.4	5.0	2.6	3.7
2. C. K.	2/8/54	26	M	54	1.65	62	+49	115	84	20	244	17.0	13.9	3.1	7.8	8.9
No. 293983	5/9/54			70	1.84	6	—	68	87	13	111	20.0	16.6	3.4	3.3	3.9
3. G. A.	8/17/54	42	M	69	1.88	96	+85	83	96	16	212	17.4	12.8	4.6	4.6	6.0
No. 297719	11/18/54			70	1.89	59	+79	78	117	16	119	17.9	13.8	4.1	2.9	4.6
	3/1/55			86	2.06	6	+ 2	55	100	11	127	20.6	15.9	4.7	2.7	6.4
4. V. F.	7/28/54	45	F	73	1.84	75	—	100	122	32	198	16.2	12.3	3.9	5.1	7.9
No. 296864	1/5/55			78	1.90	39	—	90	123	26	136	17.3	13.5	3.8	3.6	6.0
5. A. R.	6/29/54	56	F	45	1.37	60	+28	115	102	20	170	19.3	15.2	4.1	4.2	5.8
No. 296774	10/13/54			47	1.40	27	—	80	98	12	99	18.1	13.9	4.3	2.3	3.1
6. L. M.	2/22/54	63	F	38	1.25	55	—	111	113	31	156	16.5	11.0	5.5	2.8	4.3
No. 294266	6/3/54			44	1.32	—	+19	74	131	24	129	20.1	13.7	6.4	2.0	3.6
7. C. A.	4/15/54	67	M	51	1.64	78	+48	92	85	27	270	15.7	11.5	4.1	5.9	6.6
No. 247736	9/22/54			63	1.79	65	—	74	102	24	198	17.4	13.6	3.8	5.2	7.3
8. A. P.	6/7/54	74	F	35	1.25	76	—	102	98	24	140	16.3	12.5	3.8	3.6	4.9
No. 296190	10/7/54			36	1.25	42	—	90	83	22	130	14.9	10.8	4.1	3.2	3.6
Mean toxic†						68±8		104±14	102±14	24±6	192±54	17.0±1.2	12.9±1.4	4.0±0.7	4.9±1.9	6.5±1.9
Mean R_x‡						28±22		75±12	104±16	18±7	133±29	18.4±2.0	13.9±1.8	4.4±1.1	3.1±1.0	4.7±1.6
% Change						−59%		−28%	−2%	−25%	−31%	+8%	+8%	+10%	−37%	−28%
P Value						<0.001		<0.01	<0.7	<0.01	<0.01	<0.1	<0.2	<0.2	<0.05	<0.1
9. F. K.	4/21/54	36	M	50	1.57	49	+71	108	87	14	223	15.7	11.0	4.7	4.7	5.5
No. 295446	9/29/54			49	1.57	26	+25	96	90	18	155	17.7	13.4	4.3	3.6	4.4
P Value + F. K.								<0.001		<0.02	<0.01				<0.02	<0.1
10. L. K.	7/20/54	22	F	46	1.40	71	+46	115	121	23	179	14.4	10.1	4.3	4.2	6.1
No. 296992																
Ave. of 9 normals		39±13	2F/7M	73	1.85			80±16	95±8	18±4	136±20	17.1±1.5	13.2±1.3	3.9±0.5	3.6±0.5	4.6±1.0
% Diff. from thyrotoxics								−30%	−7%	−33%	−41%	0	+2%	−3%	−36%	−41%
P Value								<0.01	<0.2	<0.05	<0.02	—	—	—	<0.05	<0.02

* S.A.—Body surface area in square meters.
Syst. MABP—Systemic arterial blood pressure mean in mm. Hg.
P.A. MABP—Pulmonary arterial blood pressure mean in mm. Hg.
O_2 cons.—Oxygen consumption in cc./square meter body surface.
Art. O_2—Arterial oxygen content in cc./100 cc. of blood.
M. ven. O_2—Mixed venous (pulmonary artery) oxygen content in cc./100 cc. of blood.
Δ A-V O_2—Arterial-mixed venous oxygen difference in cc./100 cc. of blood.
Cardiac index—Cardiac output per square meter body surface area.
L.V. work index—Left ventricular work in Kg. meters/min./square meter body surface area.

TABLE I—*Continued*

Patient	Date	Age	Sex	R.V. work index	Total periph. resist.	Total pulm. resist.	Cor. sinus O_2 *Vol. %*	Δ A-CS O_2 *Vol. %*	Cor. blood flow	CMR O_2	CMR O_2/beat	C.V.R.	Index of effic.	Body R.Q.
1. W. H.	5/12/54	11	M	2.1	843	202	4.0	12.7	106	10.9	0.09	0.9	0.82	0.71
No. 288722	8/11/54			0.3	2,443	188	4.2	14.2	54	4.8	0.07	1.8	0.77	0.79
2. C. K.	2/8/54	26	M	2.1	523	124	5.9	11.1	108	12.0	0.10	0.7	0.74	0.76
No. 293983	5/9/54			0.6	1,167	174	9.0	11.0	49	5.4	0.08	1.7	0.72	0.90
3. G. A.	8/17/54	42	M	1.0	884	203	5.2	12.2	—	—	—	—	—	0.81
No. 297719	11/18/54			0.6	1,704	233	4.8	13.1	130	17.0	0.22	1.3	0.27	0.69
	3/1/55			0.4	1,439	158	5.4	15.0	56	8.4	0.15	1.9	0.76	0.76
4. V. F.	7/28/54	45	F	2.2	1,048	275	4.4	11.9	144	17.1	0.17	0.9	0.46	0.73
No. 296864	1/5/55			1.3	1,442	305	4.8	12.8	122	15.6	0.13	1.1	0.38	0.76
5. A. R.	6/29/54	56	F	1.1	1,424	279	5.9	13.4	87	11.6	0.10	1.0	0.50	0.74
No. 296774	10/13/54			0.4	2,440	299	6.5	11.4	60	6.8	0.09	1.6	0.46	0.69
6. L. M.	2/22/54	63	F	1.2	2,500	696	4.3	12.9	85	10.9	0.10	1.3	0.39	0.74
No. 294266	6/3/54			0.7	3,792	728	4.0	16.1	45	7.2	0.10	2.8	0.50	0.76
7. C. A.	4/15/54	67	M	2.1	678	224	4.5	11.2	92	10.1	0.11	0.9	0.65	0.71
No. 247736	9/22/54			1.9	879	222	6.1	11.3	64	7.2	0.10	1.6	1.00	0.93
8. A. P.	6/7/54	74	F	1.2	1,729	422	5.1	11.4	71	8.1	0.08	1.3	0.60	0.76
No. 296190	10/7/54			0.9	1,679	445	4.4	10.2	63	6.4	0.07	1.3	0.56	0.77
Mean toxic†				1.6±0.6	1,306±661	307±178	4.9±0.7	12.2±0.9	103±24	12.2±3.2	0.12±0.05	1.0±0.2	0.55±0.18	0.73±0.03
Mean R‡				0.8±0.5	1,910±940	315±191	5.6±1.7	12.8±2.1	64±24	7.7±3.4	0.10±0.03	1.7±0.5	0.64±0.20	0.80±0.03
% Change				−50%	+32%	+3%	+14%	+5%	−38%	−37%	−17%	+70%	+14%	+10%
P Value				<0.01	<0.05	<0.6	<0.2	<0.4	<0.01	<0.01	<0.05	<0.01	<0.3	<0.1
9. F. K.	4/21/54	36	M	0.9	950	153	4.7	11.0	72	7.9	0.07	1.2	0.70	0.74
No. 295446	9/29/54			0.9	1,273	255	5.6	12.4	100	12.4	0.11	1.0	0.35	0.76
P Value + F. K.				<0.02	<0.05	<0.3			<0.02	<0.05	<0.1	<0.05		
10. L. K.	7/20/54	22	F	1.3	1,657	315	4.8	9.5	98	9.3	0.08	1.2	0.66	0.79
No. 296992														
Ave. of 9 normals		39±13	2F/7M	0.9±0.2	1,200±165	220±53	5.3±1.5	12.1±1.8	74±17	8.6±2.2	—	1.3±0.4	—	0.78±0.10
% Diff. from thyrotoxics				−78%	−9%	−40%	−8%	−1%	−39%	−42%	—	+23%	—	+6%
P Value				<0.02	<0.6	<0.2	<0.1	—	<0.05	<0.05	—	<0.1	—	—

R.V. work index—Right ventricular work in Kg. meters/min./square meter body surface area.
Total periph. resist.—Total peripheral resistance in dynes/cm.$^{-5}$/second.
Total pulm. resist.—Total pulmonary resistance in dynes/cm.$^{-5}$/second.
Cor. sinus—Coronary sinus oxygen content in cc./100 cc. of blood.
Δ A-CS O_2—Arterial-coronary sinus oxygen content in cc./100 cc. of blood.
Cor. blood flow—Coronary blood flow in cc./100 gm./minute.
CMR O_2—Cardiac metabolic rate for oxygen in cc./100 gm. of muscle/minute.
CMR O_2/beat—Cardiac metabolic rate for oxygen in cc./100 gm. of muscle/minute, divided by the cardiac rate.
C.V.R.—Coronary vascular resistance (mean arterial blood pressure in mm. Hg divided by coronary blood flow in cc./100 gm./minute).
Index of effic.—Left ventricular work in Kg. meters/minute divided by CMR_{O_2}.
† The mean of observations on the patients when thyrotoxic.
‡ The mean of observations on the patients after treatment.

estimation of left ventricular weight should any change in myocardial weight have occurred during the interim between studies (average 3.9 months). Without such specific and presently unobtainable information, no speculation concerning relative efficiency of normal and thyrotoxic heart muscle is justified. Although there was a slight increase in body respiratory quotient in the second as compared to the first study, this change was not statistically significant. This trend may be related to the fact that these patients were studied at the end of a 15-hour fasting period, and owing to their hypermetabolism, had more nearly exhausted their glycogen stores than at the time of the second study.

When both treated and untreated thyrotoxic patients are grouped, significant direct correlations emerge between I^{131} uptake and the cardiac index ($p < 0.05$), left ventricular work index ($p < 0.01$), and coronary blood flow ($p < 0.02$). The relation between I^{131} uptake and myocardial oxygen uptake was not significant ($p < 0.1$). Since the oxygen consumption per square meter is the numerator of the cardiac index equation, it is not valid to calculate the correlation between BMR and cardiac index; the arteriovenous oxygen difference remained constant and obviously a direct relation has to exist. A significant correlation between cardiac output and coronary blood flow existed ($p < 0.05$) with the fraction of cardiac output which perfused each 100 grams of left ventricular muscle remaining 2.1 per cent in both treated and untreated patients.

SUMMARY AND CONCLUSIONS

1. Hemodynamic observations in patients with thyrotoxicosis are reported. In eight patients studies were made before and after treatment. The results were compared with those from nine normal subjects.

2. Evidence is presented that the hypermetabolic state of thyrotoxicosis includes the myocardium since there is a significant increase in coronary blood flow and cardiac metabolic rate for oxygen.

3. Following specific treatment, the cardiac output, cardiac work, coronary blood flow and myocardial oxygen consumption returned to normal, and the coronary vascular resistance increased.

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THE FATE OF SODIUM GLUCURONATE AND GLUCURONOLACTONE IN MAN [1]

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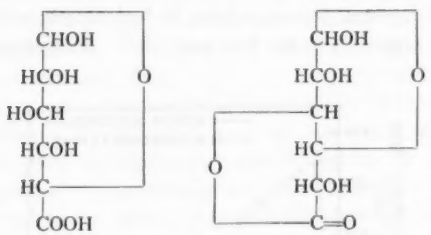
Glucuronic acid occupies an important place in the body economy. A large number of toxic substances as well as many normal metabolites are excreted as conjugates with glucuronic acid (1). The glucuronic acid moiety occurs in the mucopolysaccharides including hyaluronic acid, chondroitinsulfuric acid, mucoitinsulfuric acid, heparin and several antigenic polysaccharides (2). The use of glucuronolactone has been suggested in the treatment of arthritis (3, 4) and in the management of recurrent nephrolithiasis (5).

Glucuronic acid occurs both as the free acid (I) and as a lactone (II). These two forms differ chemically in another way; the hemiacetal ring as



(I) (II)

well as the lactone ring are five-membered in glucuronolactone, while the free acid has a six-membered pyranose structure (6, 7). It would not be unreasonable, therefore, to expect glucuronolactone and free glucuronic acid, or its salts, to be metabolized in different fashion. In the feeding experiments which have been reported (8–12), substantial amounts of reducing substances have been found in the urine, and naphthoresorcinol reactive substances have appeared in the blood and urine after the ingestion of glucuronolactone. Quick (13), however, was not able to demonstrate any urinary reducing substances after the ingestion of 3 g. of sodium glucuronate and only small amounts of reducing substances were found after the ingestion of 10 g. or more. The present study was undertaken to determine the comparative fates of sodium glucuronate and glucuronolactone in man.

METHODS

Commercial sodium *d*-glucuronate monohydrate and *d*-glucuronolactone [3] were given to apparently healthy young adults in two series of studies. Some glucuronolactone and sodium glucuronate was recrystallized, the lactone from absolute ethanol and the sodium salt from 40 per cent ethanol in water, for use as standards. For intravenous administration, recrystallized sodium glucuronate or glucuronolactone was given as a 2½ per cent solution in 2½ per cent dextrose in water over a 30-minute period.

In the first group of observations, 6 g. of either the sodium salt or lactone, divided into three equal doses, was ingested daily. The total 24-hour urinary glucuronic acid was determined prior to the period of administration and again during the fourth day of administration. In the second set of studies, urine and plasma glucuronic acid and urine pentose were determined prior to, and at intervals after the administration of a single dose of either the lactone or the sodium salt. In addition, the total amount of glucuronic acid excreted above the basal excretion level during the 12 hours after the administration of the test dose was calculated.

Total glucuronic acid was determined photometrically by means of the color reaction with carbazole (14). One ml. of diluted urine or a protein free filtrate of plasma containing less than 100 γ glucuronic acid was added to 6 ml. concentrated sulfuric acid and heated in a boiling water bath for 20 minutes. The mixture was quickly cooled to room temperature and 0.2 ml. of a 0.1 per cent solution of carbazole in ethanol was added. After several minutes, the absorption was measured at 530 mμ. in a Beckman model DU spectrophotometer using a blank similarly prepared except that 0.2 ml. ethanol was substituted for the carbazole solution. In recovery experiments in which 50.0 mg. glucuronolactone was added to each of ten random urine specimens, the increase in glucuronic acid determined by this method corresponded to 93.6 ± 3.1 per cent of the added glucuronolactone. In a similar experiment using sodium glucuronate, the recovery was 98.6 ± 4.2 per cent. The color reaction with

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[3] Generously supplied by Dr. Neal E. Artz, Corn Products Refining Co., Argo, Illinois.

TABLE I

Effect of oral sodium glucuronate and glucuronolactone on 24-hour urinary glucuronic acid excretion

Subject	Sodium glucuronate		Glucuronolactone	
	Baseline	6 g./day	Baseline	6 g./day
	mg.	*mg.*	*mg.*	*mg.*
B. S.	433	542	421	1,339
J. R.	444	656	462	1,573
G. W.	492	607	472	1,347
R. D.	460	638	487	1,290

naphthoresorcinol (15, 16) was used in preliminary studies, but it was found to be less specific and reproducible than the carbazole method of Dische.

Pentoses were determined by the procedure of Roe and Rice (17).

Glucuronolactone and the ester glucuronides react with hydroxylamine at pH = 4.5 to form hydroxamic acids, while glucuronic acid and its salts and the acetal ("ether") glucuronisides do not react with hydroxylamine. The hydroxamic acids are determined photometrically after the addition of ferric chloride (18). This method was used to assess the amount of glucuronic acid excreted in the lactone form.

RESULTS

The excretion of glucuronic acid in the urine before and after the daily ingestion of 6 g. of sodium glucuronate or glucuronolactone is given in Table I. The average increment in the total urinary glucuronic acid was 156 mg. per 24 hours on the fourth day of ingestion of sodium glucuronate, and 927 mg. per 24 hours after ingestion of the lactone. In the case of the lactone, the additional urinary glucuronic acid represents an average of 15.4 per cent of the amount ingested, while an average of only 2.6 per cent of the ingested sodium salt appears in the urine.

After the ingestion of a single dose of 15 g. glucuronolactone, a prompt rise in the plasma glucuronic acid and in the urinary glucuronic acid and pentose excretion was observed. Only slight increases were observed after similar oral doses of sodium glucuronate. Typical results obtained in one subject are illustrated in Figure 1. It was found that the bulk of the urinary glucuronic acid recovered after the ingestion of the lactone did not react with hydroxylamine, and appears, therefore, to be in the free acid form (Table II). The results of the intravenous administration of 5 g. of sodium glucuronate and of glucuronolactone in one subject are shown in Figure 2. In contrast to the small amount of an oral dose which appeared in the urine, an intravenous dose of sodium glucuronate was almost quantitatively excreted. The urinary glucuronic acid recoveries above basal excretion during the 12 hours after a single dose of either sodium glucuronate or the lactone are given in Table III.

DISCUSSION

The observation of but slight increments in the plasma and urine glucuronic acid levels after ingestion of sodium glucuronate in contrast to the prompt and substantial increases found after the ingestion of glucuronolactone is consistent with the concept that the free acid form and salts of glucuronic acid are poorly absorbed from the gastrointestinal tract.

Almost all of the glucuronic acid which appears in the urine after ingestion of glucuronolactone is unreactive with hydroxylamine. The excreted glucuronic acid is presumed to be that portion of the ingested lactone which is hydrolyzed within the organism to the free acid (19). Parenterally

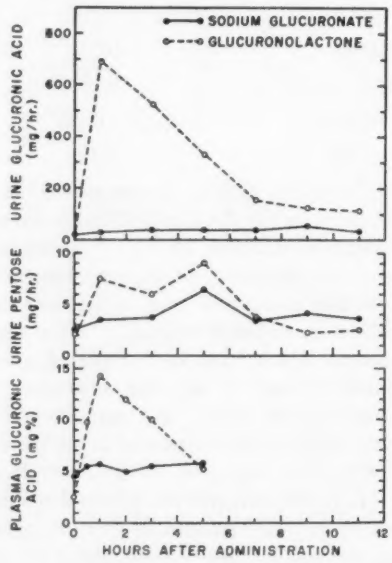

FIG. 1. A COMPARISON OF THE URINE GLUCURONIC ACID AND PENTOSE AND THE PLASMA GLUCURONIC ACID AFTER THE ORAL ADMINISTRATION OF 15.0 G. SODIUM *d*-GLUCURONATE AND AFTER A SIMILAR DOSE OF *d*-GLUCURONOLACTONE (L. W.)

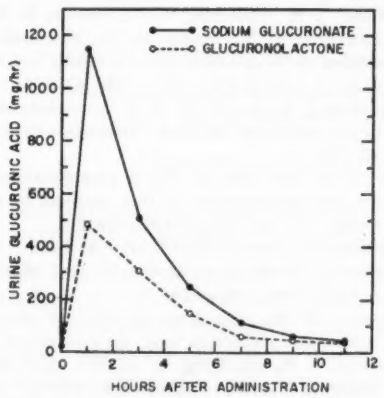

FIG. 2. A COMPARISON OF THE URINE GLUCURONIC ACID AFTER THE INTRAVENOUS ADMINISTRATION OF 5.0 G. SODIUM *d*-GLUCURONATE AND AFTER A SIMILAR DOSE OF *d*-GLUCURONOLACTONE (R. D.)

administered sodium glucuronate is promptly excreted in the urine.

The observations of this study are in agreement with the findings of recent tracer studies in guinea pigs and rats. Douglas and King (20, 21), and Packham and Butler (22, 23) found about one-half of parenterally administered glucuronolactone labeled with radiocarbon in the urine, and about one-third in the expired carbon dioxide of their animals. A parenteral dose of labeled sodium glucuronate was almost quantitatively excreted in the urine and no label was found in the expired carbon dioxide.

Enklewitz and Lasker (24) found in cases of essential pentosuria that ingestion of *d*-glucuronolactone resulted in an increase in the urine pentose. Touster, Hutcheson, and Reynolds (25) discovered that normal as well as pentosuric subjects excrete pentose after ingestion of glucuronolactone. In normal subjects, however, only a small amount of pentose appears in the urine. In both reports, the pentose was identified as *l*-xylulose. Indeed, in these studies a slight pentosuria was observed after ingestion of glucuronolactone.

It appears that *d*-glucuronolactone is absorbed from the gastro-intestinal tract and is metabolically active. Sodium *d*-glucuronate is poorly absorbed and does not enter into the metabolic reactions of the organism but is promptly excreted in the urine.

TABLE II

Total glucuronic acid and hydroxylamine reactive substances excreted after ingestion of 15 g. glucuronolactone (L. W.)

	Total urinary glucuronic acid (μEq./hr.)	Hydroxylamine reactive cmpds. (μEq./hr.)
Control	119	7.3
1 hr.	3,543	90.5
3 hr.	2,102	23.8
5 hr.	553	17.8

SUMMARY

An average of 23.4 per cent of an orally ingested dose of 15 g. *d*-glucuronolactone was excreted in the urine within 12 hours while an average of only 1.9 per cent of a similar dose of sodium *d*-glucuronate was excreted. The excreted glucuronic acid was largely in the free acid form. A prompt rise in the plasma glucuronic acid was observed after ingestion of the lactone but not after sodium glucuronate. After intravenous administration, 42 per cent of a 5 g. dose of the lactone and 80 per cent of a similar dose of sodium glucuronate was recovered from the urine. After feeding 6 g. glucuronolactone daily in divided doses for four days, the total urinary glucuronic acid increased an average of 927 mg. per 24 hours. In the case of sodium glucuronate, the increase was 156 mg.

TABLE III

Urinary glucuronic acid recovered above baseline during 12 hours after administration of sodium glucuronate or glucuronolactone

Subject	Amt. given	Route	After sodium glucuronate		After glucuronolactone	
	g.		g.	%	g.	%
L. W., 24M	15.0	Oral	0.260	(1.7)	3.536	(23.6)
J. W., 33M	15.0	Oral	0.312	(2.1)	3.730	(24.9)
R. D., 29M	15.0	Oral	0.382	(2.5)	3.906	(26.0)
C. D., 24F	15.0	Oral	0.163	(1.1)	2.846	(19.0)
J. T., 24M	15.0	Oral	0.350	(2.3)	—	—
R. D., 29M	5.0	I.V.	4.014	(80.3)	2.102	(42.1)

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THE PERMEABILITY OF CAPILLARIES TO SERUM CHOLESTEROL[1]

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The view of Man and Peters (1) that under normal conditions the capillaries are impermeable to lipids has become widely accepted. The present investigation was undertaken in order to determine to which extent cholesterol would leave the capillaries under the influence of passive congestion and what effect the initial level of cholesterol in the serum would have on the amount of cholesterol passing through the capillary wall.

REVIEW OF LITERATURE

Landis, Jonas, Angevine, and Erb (2) in studying the permeability of the capillary walls to proteins found that very low congestion pressures resulted in the filtration of small amounts of fluid but no detectable loss of protein from the blood stream. High degrees of venous congestion, however, caused the filtration of considerable amounts of fluid and protein. Geyer, Keibl, and Kölbl (3) under similar conditions observed a selective filtration of protein molecules of different sizes through the capillary wall.

Man and Peters (1) determined the amounts of protein, fatty acids, lipid phosphorus and cholesterol in the blood serum of normal persons before and after standing quietly for half an hour. They found that during standing the concentration of the lipids in the serum increased to the same extent as the concentration of proteins and concluded that the capillary wall was impermeable to lipids. Similarly, Frey (4) regarded the capillaries as impermeable to cholesterol and lipoproteins since these substances were not detected in subcutaneous edema fluid. Geyer, Keibl, and Kölbl (3) found the capillaries impermeable to the lipid bearing proteins, namely the alpha and beta globulins, unless the capillaries had been damaged by an increase in the intracapillary pressure. On the other hand, Marble, Field, Drinker, and Smith (5) concluded that the walls of the blood capillaries were slightly permeable to some lipid substances because, after an intravenous infusion of a fat emulsion, the lymph fluid showed a slight increase in the amount of total lipids but not of cholesterol.

METHODS

Landis, Jonas, Angevine, and Erb (2) produced venous stasis of one forearm by means of a blood pressure cuff in order to determine the rate of filtration of fluid and protein from the capillaries. This procedure has also been used in this study to determine the rate of filtration of cholesterol in a number of normal individuals and patients. All tests were carried out in the morning before the subjects had had breakfast. Except for three subjects attending the out-patient department all test persons had remained in bed on the morning of the test. All subjects were kept reclining for at least half an hour before the test was started. The arm to which the pressure cuff was applied was supported by pillows in a horizontal position at the level of the body with the flexor surface turned upward. Then the armlet of a blood pressure apparatus was wrapped around the arm well above the elbow. The congesting pressure of the cuff was kept at either 40, 50, 60, 70 or 80 millimeters of mercury for 30 minutes. Pre-congestion blood samples were drawn from the antecubital vein before the cuff was placed into position. Post-congestion blood samples were drawn at the end of the 30-minute test period with the congesting cuff still in position. In both samples the hematocrit value and the concentration of serum cholesterol were determined. Since the evaluation of the effect of venous congestion is based on the assumption that under test conditions erythrocytes do not pass the capillary wall some experiments in which petechiae appeared on the congested forearm have been excluded and are not mentioned in the reported series.

The relative amount of fluid lost through the capillary walls as a result of increase in intracapillary pressure was calculated from the difference in the hematocrit values in samples of blood taken before and after venous stasis by the following formula:

$$F = 100 - \frac{100 \cdot H_1}{H_2}$$

F = ml. of fluid lost from 100 ml. of whole blood.
H_1 = Hematocrit value prior to congestion.
H_2 = Hematocrit value after congestion.

[1] This investigation was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases, of the National Institutes of Health, Public Health Service (PHS A-414).

The amount of cholesterol passing from the plasma through the capillary wall was calculated by the formula used by Landis, Jonas, Angevine, and Erb (2) for the determination of protein losses:

$$\Delta Ch = \frac{Ch_1 (100 - H_1) - Ch_2 (100 - H_2 - F)}{100}$$

ΔCh = mg. of cholesterol lost from 100 ml. of whole blood

Ch_1 = Serum cholesterol level in mg. per 100 ml. prior to congestion

Ch_2 = Serum cholesterol level in mg. per 100 ml. after congestion

TABLE I

Initial level of serum cholesterol and amounts of fluid and cholesterol filtered after congestion of the forearm for thirty minutes

Subject No.	Congesting pressure applied *mm. Hg*	Ch Initial level of serum cholesterol *mg./100 ml.*	F Amount of fluid filtered from 100 ml. of blood *ml.*	ΔCh Amount of cholesterol filtered from 100 ml. of blood *mg.*
1	60	216	2.0	0
2	40	182	4.2	0
3	40	241	4.7	0
4	60	242	6.2	0
5	60	248	8.2	0
6	60	208	9.6	0
7	60	326	5.7	5.0
8	60	358	7.2	7.6
9	60	208	8.3	8.5
10	60	118	9.1	1.0
11	40	307	9.6	6.0
12	60	295	10.2	8.5
13*	50	400	10.2	14.0
14	60	278	10.2	19.3
15	60	227	10.4	5.7
16	60	364	10.7	6.0
17	60	204	11.5	3.0
18	60	234	12.1	5.0
19	60	237	13.0	3.0
20	60	235	13.1	6.0
21	60	269	13.3	8.5
22	60	218	13.7	4.5
23	60	289	14.0	12.9
24	60	259	14.3	11.0
25	40	319	14.3	30.5
26	70	214	14.7	7.3
27*	50	406	14.7	31.5
28	80	373	15.2	21.0
29	60	319	15.6	22.0
30	60	251	16.0	17.5
31	70	248	16.4	7.3
32	60	250	18.4	14.0
33	80	241	19.4	12.8
34	60	273	19.6	25.0
35	80	264	19.7	21.0
36	80	265	20.0	31.0
37	60	357	20.1	40.0
38	80	226	21.3	14.5
39*	80	430	24.6	35.0
40	80	212	25.4	16.6

* Patients with primary hypercholesteremic xanthomatosis.

The serum total cholesterol was determined by a modification of the Schoenheimer-Sperry method (6). The standard error of this method in our Laboratory has been ±9.25 mg. per 100 ml. of serum.

RESULTS

Determinations were carried out on 40 persons (Table I). Three subjects had primary hypercholesteremic xanthomatosis. The serum cholesterol value exceeded 350 mg. per 100 ml. in seven individuals. The entire group has been divided into three sub-groups according to the amount of fluid and cholesterol which passed through the capillary walls during the test procedure:

Group I. In 6 persons (No. 1–6) small amounts of fluid and no measurable amount of cholesterol were lost through the capillary walls. The loss of fluid varied from 2.0 to 9.6 ml. per 100 ml. of blood and averaged 5.8 ml. The initial values for serum cholesterol ranged from 182 to 248 mg. per 100 ml. of serum, averaging 223 mg. The congesting pressure applied in these 6 persons was either 40 or 60 mm. of mercury.

Group II. In 25 persons (No. 7–31) moderate amounts of fluid and varying amounts of cholesterol were lost through the capillary walls. The loss of fluid varied from 5.7 to 16.4 ml. per 100 ml. of blood, averaging 12.1 ml., and the loss of cholesterol varied from 1.0 to 31.5 mg. per 100 ml. of blood, averaging 10.9 mg. The initial values for serum cholesterol ranged from 118 to 406 mg. per 100 ml. of serum, averaging 278 mg. In five subjects the initial value for cholesterol was in excess of 350 mg. per 100 ml. of serum. The congesting pressure applied ranged from 40 to 80 mm. of mercury.

Group III. In 9 persons (No. 32–40) large amounts of fluid and usually also large amounts of cholesterol were lost through the capillary walls. The loss of fluid varied from 18.4 to 25.4 ml. per 100 ml. of blood, averaging 20.9 ml., and the loss of cholesterol varied from 12.8 to 40 mg. per 100 ml. of blood, averaging 23.3 mg. The initial values for serum cholesterol ranged from 212 to 430 mg. per 100 ml. of serum, averaging 280 mg. In two persons the initial value for cholesterol was in excess of 350 mg. per 100 ml. of serum. The congesting pressure applied was either 60 or 80 mm. of mercury.

A diagram designed to show the relation be-

tween fluid loss and cholesterol loss showed rather wide scattering (Figure 1). The value for a linear correlation between the losses of fluid and cholesterol (as calculated by the coefficient of correlation) proved to be slightly smaller than the value for a parabolic correlation (as calculated by the index of correlation). The difference, however, between the values for linear and parabolic regressions was found to be insignificant by the F-test. Thus a linear regression was assumed and the following equations between the loss of fluid (F), the loss of cholesterol (ΔCh) and the initial level of total cholesterol (Ch) were calculated by the method of least mean squares:

(*1*) $\Delta Ch = 1.385\,F - 6.18$

(*2*) $\Delta Ch = 1.1875\,F + 0.07515\,Ch - 24.002$

The coefficient of correlation between ΔCh and F amounted to $r = 0.71$, and the coefficient of correlation between ΔCh and Ch to $r = 0.61$. Thus, both coefficients of correlation differed significantly from $r = 0$ as checked by the Student's t-test. Applying the formula used in this test,

$$\frac{|r|}{\sqrt{1 - r^2}} \cdot \sqrt{N - 2} > t_{38}\,(= 3.65) \text{ for } 99\%$$

was calculated as $6.2 > 3.56$ for equation *1* and as $4.41 > 3.56$ for equation *2*. The coefficient of correlation between F and ΔCh (equation *2*) did not differ significantly from zero.

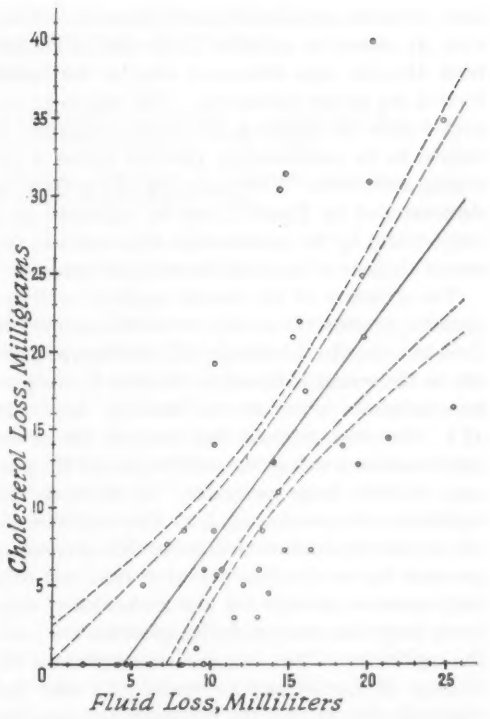

FIG. 1. CORRELATION BETWEEN THE AMOUNTS OF FLUID AND CHOLESTEROL FILTERED THROUGH THE CAPILLARY WALLS DURING CONGESTION OF THE FOREARM FOR THIRTY MINUTES

The losses of fluid and of cholesterol are calculated per 100 ml. of blood. The solid line represents an assumed linear regression while the dotted lines represent confidence limits for 95 and 99 per cent, respectively.

DISCUSSION

The amounts of fluid and of cholesterol lost from the blood stream under congesting pressure have been measured and the relations between cholesterol loss and fluid loss as well as between cholesterol loss and the initial level of serum cholesterol have been calculated by statistical means.

It appeared logical to correlate the rate of cholesterol filtration to the rate of fluid filtration because the amount of fluid filtered from the blood could be determined fairly accurately in the experiments. On the other hand, it seemed inadvisable to correlate the rate of cholesterol filtration to the amount of congesting pressure applied in spite of the fact that different degrees of pressure were applied to different subjects, because the degree of pressure applied represented only one of several physical factors affecting the rate of cholesterol filtration. Other physical factors, such as the size and length of the pores in the capillary walls, cannot be determined in living human beings. Actually, the amount of fluid filtered through the capillary walls reflects these physical factors fairly well. Therefore, it seemed justified to consider the rate of fluid filtration as the best index of the effects of all physical influences and conditions.

In 6 of 40 subjects (Group I) venous congestion resulted in no loss of cholesterol from the blood. In all these instances only small amounts of fluid were lost. In the remaining 34 subjects (Groups II and III) cholesterol in varying amounts was found to have left the serum. A significant relation existed between the rates of

fluid filtration and of cholesterol filtration. However, as shown by equation *2*, the rate of cholesterol filtration was influenced also by the initial level of the serum cholesterol. This was to be expected since the filtration rate of any substance is related to its concentration gradient across a filtrating membrane. The scattering of the data, as demonstrated by Figure 1, can be explained to a large extent by the considerable differences in the serum cholesterol levels of the subjects studied.

The structure of the normal capillary wall apparently permits the passage of small amounts of fluid but not of cholesterol. All cholesterol present in the serum is bound to proteins in lipoprotein molecules which are of relatively large size (7). One may conclude that the pore size of the intact capillary wall is too small to permit the passage of these large molecules. If, however, the capillary walls are damaged, as they undoubtedly are by the application of considerable congesting pressure for an extended period of time, not only large amounts of fluid but also molecules of relatively large size, such as the lipoproteins, can leave the capillaries. Thus, our studies supplement the findings of Landis and associates (2) who had observed that raising the intracapillary pressure increases the permeability of the capillaries to serum proteins.

SUMMARY

1. Calculation of the amounts of fluid and cholesterol lost through the capillary walls during prolonged passive congestion revealed that under experimental conditions which did not significantly alter the normal capillary filtration of water no measurable amounts of cholesterol passed the capillary wall.

2. Under experimental conditions presumably inducing alterations in the capillary walls varying amounts of cholesterol were observed to pass from the circulation. The rate of cholesterol filtration showed significant relations both to the rate of fluid filtration on the one hand and to the initial level of serum cholesterol on the other.

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THE DYNAMICS OF LEUKOPOIESIS AND LEUKOCYTOSIS, AS STUDIED BY LEUKOPHERESIS AND ISOTOPIC TECHNIQUES [1]

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Previous studies (1, 2) in normal and irradiated dogs employing the technique of leukopheresis have suggested some hitherto unknown relationships between the number of circulating leukocytes and the reserves of these cells elsewhere in the body. By way of introduction and as background for the data to be presented it is necessary to review briefly and interpret some of these findings.

The acute response to the production of severe leukopenia by leukopheresis is qualitatively similar from animal to animal and is divisible into three phases. When leukopheresis is terminated there is a delay before the peripheral white blood cell count begins to rise (phase I). Following this there occurs a linear rise in peripheral blood count over a six to eight-hour period (phase II). After a slower rise to a peak peripheral white cell count, usually 150 to 200 per cent of baseline, the white cell count stabilizes at this new level for several hours (usually 24 to 36); this last period is denoted as phase III. Phase I, or the delay period before the white blood cell count begins to rise, is not observed unless the count is reduced to 1,500 or 1,000 cells per mm^3. Less marked degrees of leukopenia, induced by slower rates of clearance of white cells from the peripheral blood, may be maintained by continued leukopheresis for several hours but are not followed by the period of sustained leukopenia (phase I). This is in spite of the removal of many more cells than would be obtained during a short, rapid leukopheresis which produces marked leukopenia. On the other hand, phase I is observed after marked (1,500 per mm^3 or less) leukopenia no matter how long leukopheresis is continued after leukopenia is induced. For these reasons phase I is thought to reflect a more rapid rate of dispersal of leukocytes to the periphery which offsets the accelerated entry of cells from the marrow into the circulation. In other words, the evidence suggests that the change from a stationary leukopenia (phase I) to a rapidly rising level (phase II) is due to a sudden alteration in the rate of distribution of leukocytes to peripheral tissues. Once this "priming" has occurred in phase I, the blood white cell count begins to rise rapidly, reflecting the accelerated entry of leukocytes into the circulating blood.

Phase II represents a period of greatly accelerated entry of leukocytes into the peripheral blood, which continues until the new maximum is reached. Rapid leukopheresis with the removal of large numbers of cells may be carried out for a prolonged period without the production of leukopenia if the procedure is instituted during phase II (see Figure 2).

Phase III represents a period of entry of leukocytes into the peripheral blood which has slowed to approximately that at time zero. Leukopheresis instituted during phase III results in the same degree of leukopenia produced by the same rate of leukopheresis as carried out initially. In most animals this type of response occurs repeatedly until about the third or fourth day. By this time acceleration in the growth of myeloid tissue has occurred so that the delay after cessation of leukopheresis is shorter and the leukocytosis develops more rapidly. The maximal white cell count in the peripheral blood after leukopheresis on the third day varies from animal to animal; in most it is similar to the level after previous leukophereses, in some it is higher and in others it is lower. All animals show a slower, progressive rise to a

[1] This work was supported by grants from Parke-Davis and Co., the U. S. Public Health Service, and Gladys F. Bowyer Fund.

[2] Markle Foundation Scholar in Medical Science.

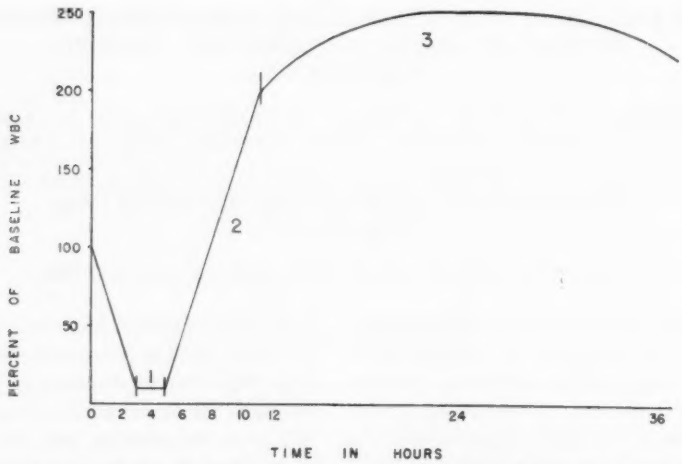

FIG. 1. SCHEMATIC REPRESENTATION OF THE TYPICAL RESPONSE OF THE CIRCULATING LEUKOCYTE COUNT TO LEUKOPENIA INDUCED BY A SINGLE LEUKOPHERESIS

Removal of leukocytes terminated at beginning of Phase 1. See text for explanation.

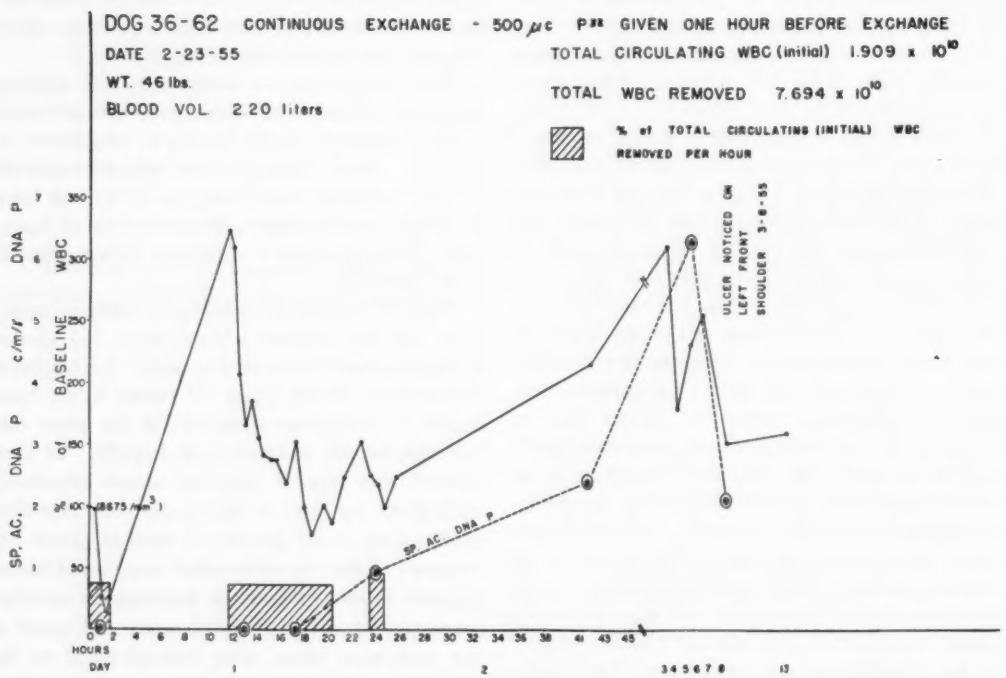
DOG 36-62 CONTINUOUS EXCHANGE - 500 μc P32 GIVEN ONE HOUR BEFORE EXCHANGE
DATE 2-23-55
WT. 46 lbs.
BLOOD VOL. 2.20 liters
TOTAL CIRCULATING WBC (initial) 1.909 x 10^10
TOTAL WBC REMOVED 7.694 x 10^10
% of TOTAL CIRCULATING (INITIAL) WBC REMOVED PER HOUR
ULCER NOTICED ON LEFT FRONT SHOULDER 3-8-55
SP. AC. DNA P c/m/γ
DNA P % of BASELINE WBC
(8675 /mm3)
SP. AC. DNA P
HOURS
DAY

FIG. 2. INDUCTION OF LEUKOPENIA BY ACUTE LEUKOPHERESIS FOLLOWED BY PROLONGED, CONTINUED LEUKOPHERESIS (OVER 8 HOURS) INSTITUTED DURING THE PERIOD OF DEVELOPING LEUKOCYTOSIS FOLLOWING THE INITIAL DEPLETION

Note that leukopenia was not induced during this time despite removal of over 7×10^{10} leukocytes. The data derived from the specific activity of white cell DNA phosphorus (expressed as counts per minute per microgram of DNA phosphorus) indicate that the removed cells were largely unlabeled.

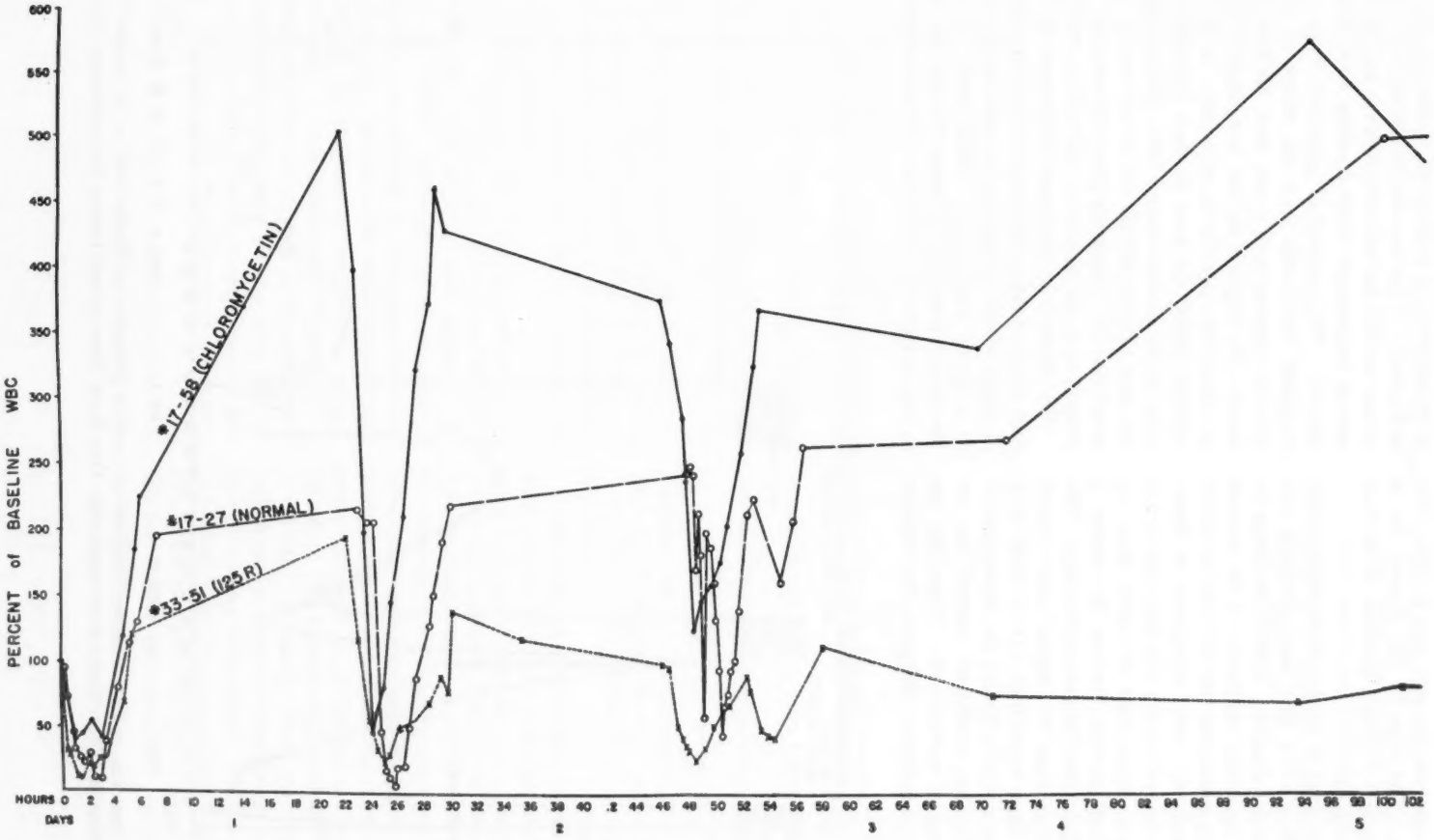

FIG. 3. COMPOSITE GRAPH SHOWING TYPICAL RESPONSES OF A NORMAL DOG, A DOG RECEIVING CHLORAMPHENICOL, AND A DOG GIVEN 125r WHOLE BODY IRRADIATION ONE HOUR BEFORE THE INITIAL LEUKOPHERESIS

Leukopheresis was carried out acutely on each dog at roughly 24-hour intervals, and hence was reinstituted during phase III. Note the delayed rise at five days in the case of the normal and chloramphenicol treated animal, a phenomenon not exhibited by the irradiated dog.

new maximum over the next two to three days (Figure 3). The slope of this delayed rise is similar in normal animals regardless of the level attained during phase II.

The morphology of the peripheral blood leukocytes and the marrow have been correlated with these quantitative changes. There is no change indicative of increasing immaturity of the marrow or circulating leukocytes until the third or fourth day. Undoubtedly some stimulation to leukopoiesis has occurred during this three-day period.

These data suggest that the *initial* acute response to leukopheresis involves the release of preformed leukocytes from areas of storage. This initial response does not depend upon growth processes. Dogs exposed to LD-50 whole body x-ray behave similarly, though the leukocytosis is quantitatively less, until their marrow has become depleted of myeloid cells. Even after the development of radiation leukopenia, the response to leukopheresis is qualitatively similar, as shown in Figure 5. A fundamental relationship seems to prevail between the leukocyte reserves and the degree of leukocytosis which develops after leukopheresis. The removal of leukocytes from the peripheral blood calls forth the release of cells from the marrow until a new level has been attained. The height of the new level depends upon the size of the reservoir of leukocytes. In the irradiated animal the level becomes progressively lower as the marrow reserve falls. Nevertheless, the same pattern prevails and leukocytes are released into the circulating blood in some sort of proportion to the number of cells held in reserve.

The reserves of preformed leukocytes in the body assume fundamental importance as the source of supply of cells taking part in acute leukocytosis or pyogenic reactions of various sorts. It is, therefore, important to delineate the size and site of such reserves of leukocytes. There are essen-

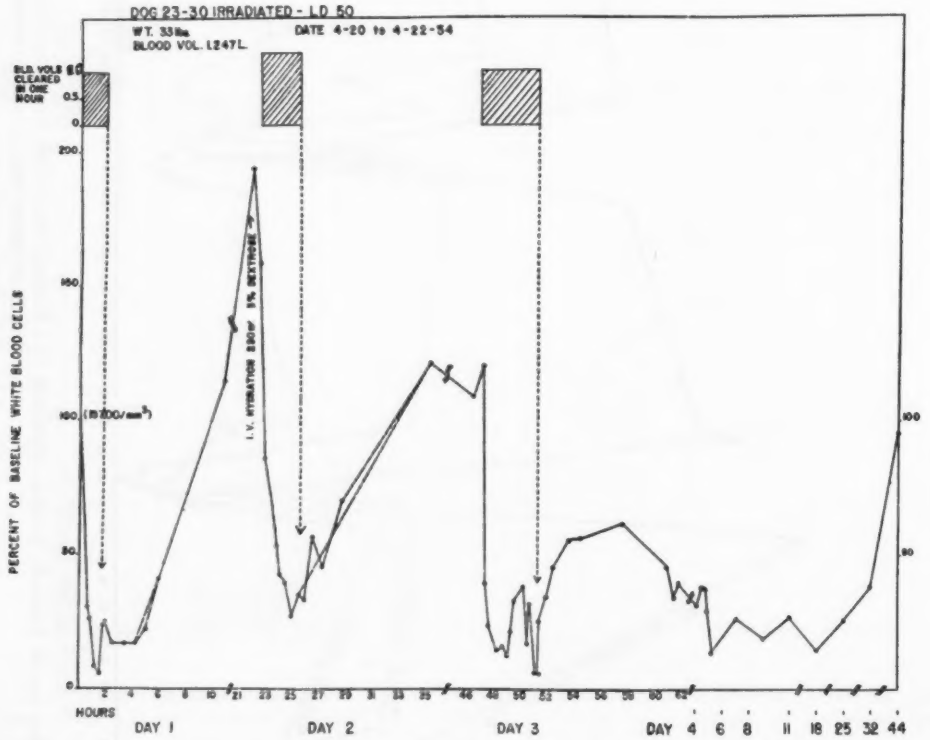

FIG. 4. RESPONSE TO LEUKOPHERESIS REPEATED AT 24-HOUR INTERVALS FOR THREE DAYS IN AN ANIMAL EXPOSED TO LD-50 WHOLE BODY IRRADIATION ONE HOUR PRIOR TO THE INITIAL LEUKOPHERESIS

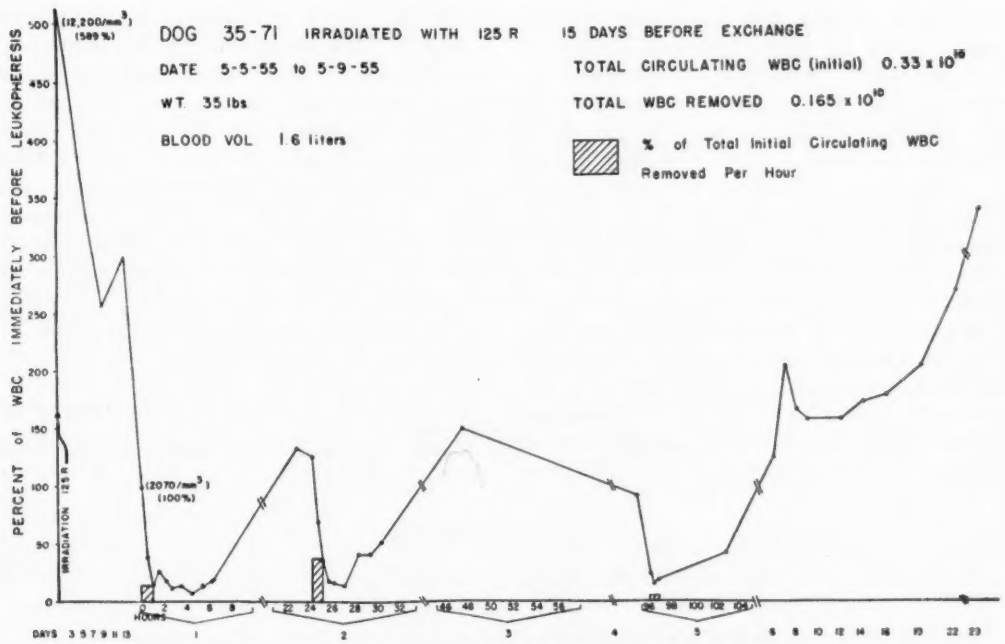
DOG 35-71 IRRADIATED WITH 125 R 15 DAYS BEFORE EXCHANGE
DATE 5-5-55 to 5-9-55
TOTAL CIRCULATING WBC (initial) 0.33 x 10^10
WT 35 lbs
TOTAL WBC REMOVED 0.165 x 10^10
BLOOD VOL 1.6 liters
% of Total Initial Circulating WBC Removed Per Hour
PERCENT of WBC IMMEDIATELY BEFORE LEUKOPHERESIS
IRRADIATION 125 R
(12,200/mm³) (589%)
(2070/mm³) (100%)
HOURS
DAYS

FIG. 5. LEUKOPHERESIS OF AN ANIMAL RENDERED LEUKOPENIC BY 125r WHOLE BODY X-RAY TWO WEEKS PREVIOUSLY

Note that the level of circulating white cells rises after each leukopheresis to a level exceeding baseline, although the rise is much more delayed than in a normal animal.

tially three potential sites of such a "ready reserve": 1) Leukocytes sequestered in various compartments of the vascular bed, 2) leukocytes held within the marrow organ, and 3) leukocytes which have migrated out of the vascular bed (peripheral tissues).

It is well known that acute fluctuations in the number of circulating leukocytes occur following hemodynamic alterations in such organs as the lung and spleen. Although the participation of such factors in the response to leukopheresis cannot be entirely eliminated, the uniformity and magnitude of the response in anesthetized animals makes unlikely such events as the leukocytosis of splenic contraction as playing an important role. Epinephrine intravenously did not alter the response (2), and the results in one splenectomized dog (Figure 6) did not differ significantly from normal.

The size of the marrow leukocyte pool has been estimated to be as much as 100 times that of the circulating blood (exclusive of marrow) (3, 4). Certainly our earlier experiments in irradiated dogs (1, 2) suggest there are sufficient numbers in the marrow to replace those circulating many times over.

Aside from the marrow the main source of leukocytes to be considered is the extramedullary, extravascular mass of leukocytes. Osgood, for example, (5) estimates that the numbers of leukocytes in such areas are so vast as to be many times the numbers in the marrow and blood. Whether leukocytes which have migrated out of the vascular tree can reenter in sufficient numbers to affect the acute response to leukopheresis is unknown. However, this appears improbable from previous work (2). A heavily irradiated dog, rendered leukopenic by repeated leukopheresis so that fixed agranulocytosis was present showed large collections of leukocytes in peripheral areas of infection in the lung and elsewhere. These leukocytes did not reenter the circulation during or after leukopheresis.

The present experiments were directed pri-

marily at the question of the source of those leukocytes contributing to the leukocytosis following leukopheresis. For this purpose the rate of entrance of newly formed leukocytes from the marrow has been studied, employing P^{32} incorporation into leukocyte DNA. Previous works by Kline and Cliffton (6) and Ottesen (7) have indicated the feasibility of this method for determining the mean age, life span and turnover rate of leukocytes. Both groups of investigators noted a delay period of four to six days before labeled myeloid leukocytes appeared in the circulation, attributed to the time required for cells capable of synthesizing DNA at the time of maximum labeling to mature to the point of being released. These workers utilized modifications of the Schmidt-Thannhauser principle of nucleic acid separation. Unfortunately, we have been unable to obtain adequate yields of DNA phosphorus uncontaminated by phosphorus from other sources using this method in the study of dog leukocytes. Similar difficulties have been encountered by others (8). Consequently, a different technique of DNA extraction developed by Dr. Norman Simmons[3] has been employed. The DNA obtained from leukocytes by this method is highly purified, as determined by spectroscopic, ultracentrifugal, x-ray diffraction, and chromatographic techniques.

The leukocyte response to leukopheresis involves myeloid leukocytes almost exclusively (1, 2). Lymphoid cells generally decrease so that the vast majority of leukocytes are of the neutrophilic

[3] Chief, Enzyme Chemistry Section, UCLA Atomic Energy Project.

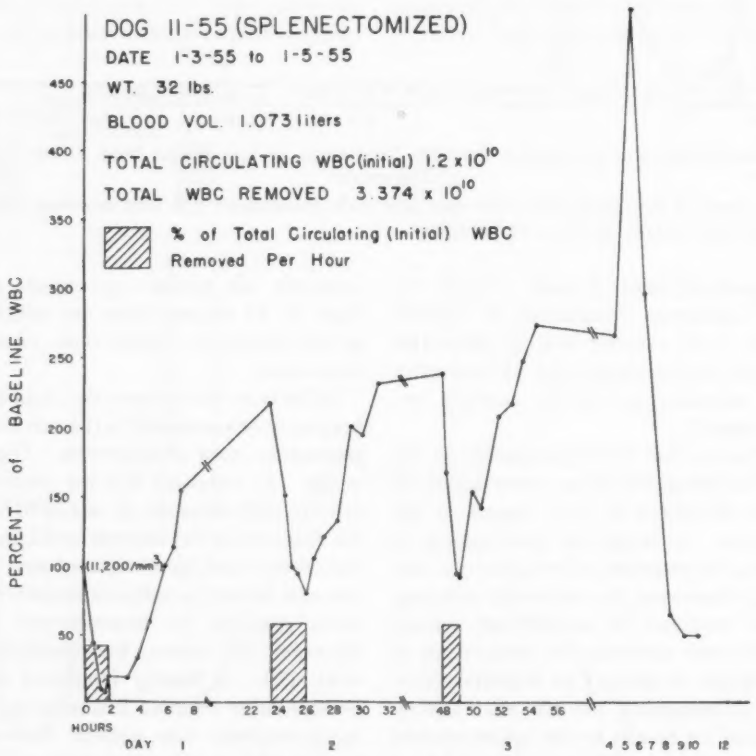

FIG. 6. THE LEUKOCYTE RESPONSE OF A SPLENECTOMIZED DOG TO REPEATED LEUKOPHERESIS

The pattern differs in no way from normal. Large numbers of nucleated red cells appeared after the initial leukopheresis; the white cell determinations were corrected for this.

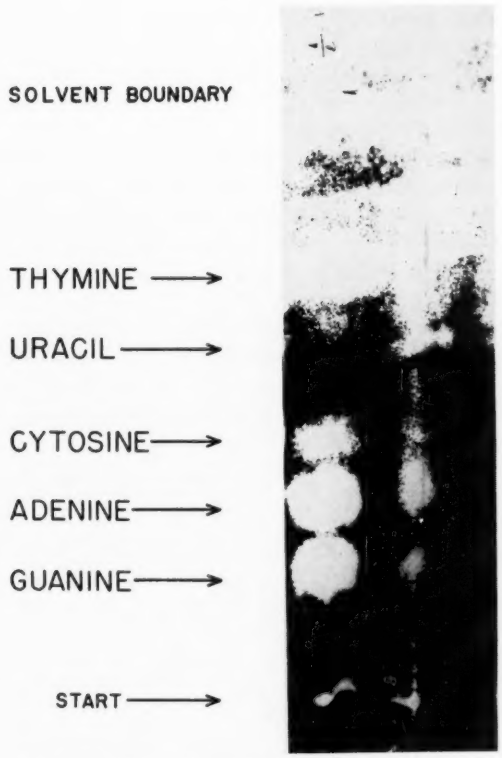

FIG. 7. PAPER CHROMATOGRAM SHOWING PURITY (LEFT) OF DOG LEUKOCYTE DNA OBTAINED BY METHOD DESCRIBED IN TEXT AS COMPARED TO VIRUS PREPARATION USING SCHMIDT-THANNHAUSER TECHNIQUE (RIGHT).

variety. No attempt has been made to obtain more complete separation of lymphocytes and neutrophiles.

METHODS

Leukocyte suspensions were obtained from dogs. Siliconed glassware was used throughout and the entire procedure was carried out at 4° C. In animals subjected to leukopheresis, sufficient numbers of leukocytes were obtained so that separation by simple centrifugation was adequate. In the case of less rich sources, leukocytes were separated from whole blood by means of dextran (Commercial Solvents, av. molecular wt. 120,000). Fifteen ml. of 6 per cent dextran per 25 ml. blood was found to give optimal separation. After sedimentation of the red cells the supernatant plasma was centrifuged at 800 to 1,000 r.p.m. for 15 minutes in an International 2 centrifuge to pack the leukocytes and leave the platelets in suspension. After washing twice with saline the leukocytes were diluted to a specific volume, mixed thoroughly and counted in quadruplicate. The theoretical amount of DNA in the cell suspension could thus be calculated.

The leukocytes were then washed with 1 per cent citric acid until the supernate was clear and then homogenized for 5 minutes in 1 per cent citric acid. They were then washed twice, or until the supernatant fluid was clear, with 0.2 per cent citric acid and twice with cold saline. The separated nuclei were subjected to a procedure involving the dissociation of nucleoprotein with 30 per cent sodium xylene sulfonate at pH 7, deproteinization by dilution, filtration, and adjustment of pH to 3.8 and precipitation of the DNA from neutral solution with isopropyl alcohol. The details of this procedure will be published subsequently by Dr. Simmons and will be the subject of a separate report concerning leukocyte nucleic acids from this laboratory.

The yield of DNA was determined by the diphenylamine reaction and by ultraviolet spectroscopy using pure DNA supplied by Dr. Simmons as standard. Organic and inorganic phosphorus determinations were made on each specimen and in all instances the former checked closely with the yield of DNA. Inorganic phosphorus amounted to less than 10 per cent and the counts of radioactivity were corrected for the small amount present. RNA contamination, as determined by the orcinol reaction, was less than 5 per cent. Quantitative chromatography [4] showed negligible RNA contamination on selected samples. An example of a chromatogram is shown in Figure 7.

The P^{32} employed was obtained from Abbott Laboratories. P^{32} counting was carried out in the Radioisotope Section, Research Division, Sawtelle Veterans Administration Hospital using a Traceriab Superscaler and a TGC-2 Geiger-Muller Tube with a statistical reproducibility of ± 1 to 2 per cent. All values have been corrected for decay.

P^{32} was administered as disodium phosphate intravenously in the amount of 500 μc. regardless of the size of the animal. (The dogs ranged in weight from 30 to 50

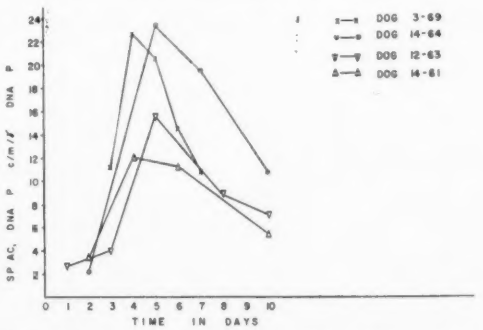

FIG. 8. SPECIFIC ACTIVITY OF DNA PHOSPHORUS IN LEUKOCYTES SAMPLED AT INTERVALS AFTER ADMINISTRATION OF 500 MICROCURIES P^{32} AT TIME 0 TO FOUR NORMAL DOGS

These animals were not leukopheresed.

[4] Dr. Gian L. Turco, Research Fellow, California Institute of Technology, graciously performed these studies for us.

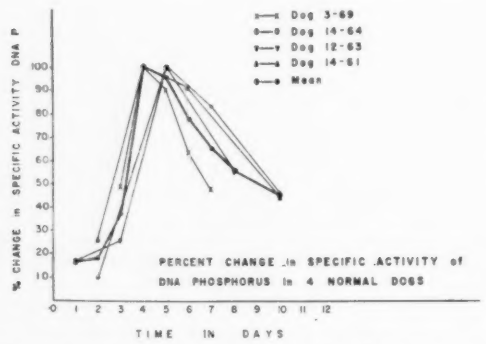

FIG. 9. SAME DATA SHOWN IN FIGURE 8 PLOTTED AS PER CENT OF THE MAXIMUM SPECIFIC ACTIVITY

lbs.) Those animals who were not subjected to leukopheresis were bled from the jugular vein under sterile conditions, into ACD-dextran solution. After separation of the leukocytes, the red cells were readministered at the time of the next bleeding. All animals withstood the procedure without showing ill effects. No hematologic depression was noticed. Although it is most probable that the amount of irradiation delivered by the P^{32} has some effect on the processes of cellular growth, the use of the technique in acute studies of differences in entry rate, distribution, and source of leukocytes should be valid.

RESULTS

Figures 8 and 9 show the results in four normal dogs whose leukocytes were sampled at intervals after administration of the isotope. These dogs were not subjected to leukopheresis. The curves are not comparable from animal to animal in terms of the amount of P^{32} incorporated into DNA since no attempt was made to administer P^{32} on a weight or blood volume basis. However, the form of the curve for each animal is similar. The mean curve (Figure 9) resembles those observed by others in humans. The rise in specific activity is earlier than in human experiments and the logarithmic curve reaches its maximum on the fourth to fifth day, followed by a brief plateau. Sufficient observations have not been made to describe fully the descending portion of the curve and for the purposes of these experiments emphasis has been placed on the rising portion of the curve as showing the time of appearance of labeled cells in the circulating blood. Calculations of mean cell age and survival time have not been attempted from these limited data, but they suggest a shorter life span for dog leukocytes than that reported for human white cells (6, 7).

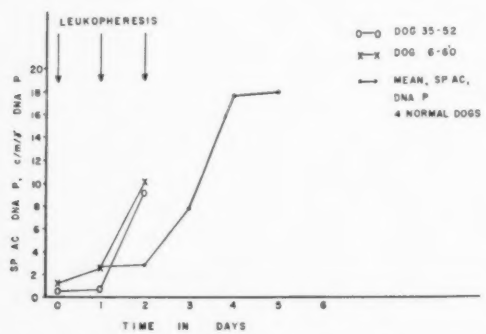

FIG. 10. ACCELERATION IN THE RATE OF ENTRANCE OF LEUKOCYTES CONTAINING DNA LABELED WITH P^{32}, 500 μc. P^{32} ADMINISTERED AT TIME 0

Leukopheresis carried out in dog 35–52 and 6–60 at indicated points resulting in the typical normal leukocyte response.

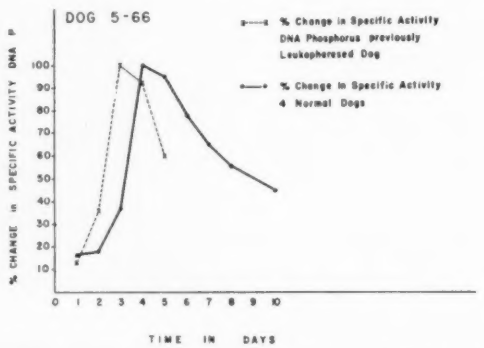
DOG 5-66
% Change in Specific Activity DNA Phosphorus previously Leukopheresed Dog
% Change in Specific Activity 4 Normal Dogs
% CHANGE in SPECIFIC ACTIVITY DNA P
TIME IN DAYS

FIG. 11. THE RATE OF APPEARANCE OF LEUKOCYTES WITH P^{32} LABELED DNA INTO THE PERIPHERAL BLOOD IN AN ANIMAL LEUKOPHERESED FOR THREE DAYS PRIOR TO ADMINISTRATION OF 500 μc. P^{32} INTRAVENOUSLY AS COMPARED TO THE CURVE FOR NORMAL DOGS NOT SUBJECTED TO LEUKOPHERESIS

If leukopheresis is carried out within two hours after administration of P^{32} the removed cells contain no labeled DNA. Furthermore the cells removed during leukopheresis 24 hours later are still unlabeled, even if large numbers of cells are removed, as is shown in Figure 2. However, repeated leukopheresis does lead to some acceleration in the time of appearance of significantly labeled leukocytes, as is shown in Figure 10. These results are compatible with a large marrow pool of mature leukocytes which precede the entry of those cells which incorporated P^{32} during the

process of DNA synthesis. The same type of data is shown in more detail in Figure 2.

Figure 11 shows the curve of specific activity of DNA phosphorus of a normal dog previously subjected to leukopheresis for 3 days before giving the isotope. The maximum of specific activity occurred 24 hours earlier than that of normal dogs not subjected to leukopheresis which suggests the more rapid release of leukocytes from the marrow at this time. This would be expected from the stimulation of leukopoiesis brought about by leukopheresis on three successive days.

Leukopheresis was carried out in three normal animals at a time when those leukocytes whose DNA was maximally labeled with P^{32} were still in the marrow but shortly before they would be normally released in significant numbers. These dogs were leukopheresed on the third day after being given 500 μc. P^{32} intravenously. At this point those cells coming from the marrow should have a high specific activity, while cells which were in peripheral areas of storage should contain negligible amounts of P^{32} labeled DNA. Because of this difference in degree of labeling of marrow and peripheral leukocyte pools it should be possible to determine the source of leukocytes contributing to the response to leukopheresis.

Figures 12 and 13 show the results in two of these animals. The third showed an identical response to Figure 13. All dogs showed a relatively low specific activity of leukocyte DNA P in those cells removed during the first leukopheresis. There was a marked rise in specific activity of circulating leukocyte DNA P during the next few hours when the leukocyte count rose after cessation of leukopheresis. It is clear, therefore, that a high percentage of the cells contributing to phase II came from the labeled marrow leukocyte pool. Both dogs showed a fall in specific activity of leukocyte DNA P from this peak the next day. Dog 20–72 (Figure 12) showed very little change in specific activity incident to the second or third leukopheresis. However, Dog 36–70 (Figure 13), after the second leukopheresis, showed a repetition

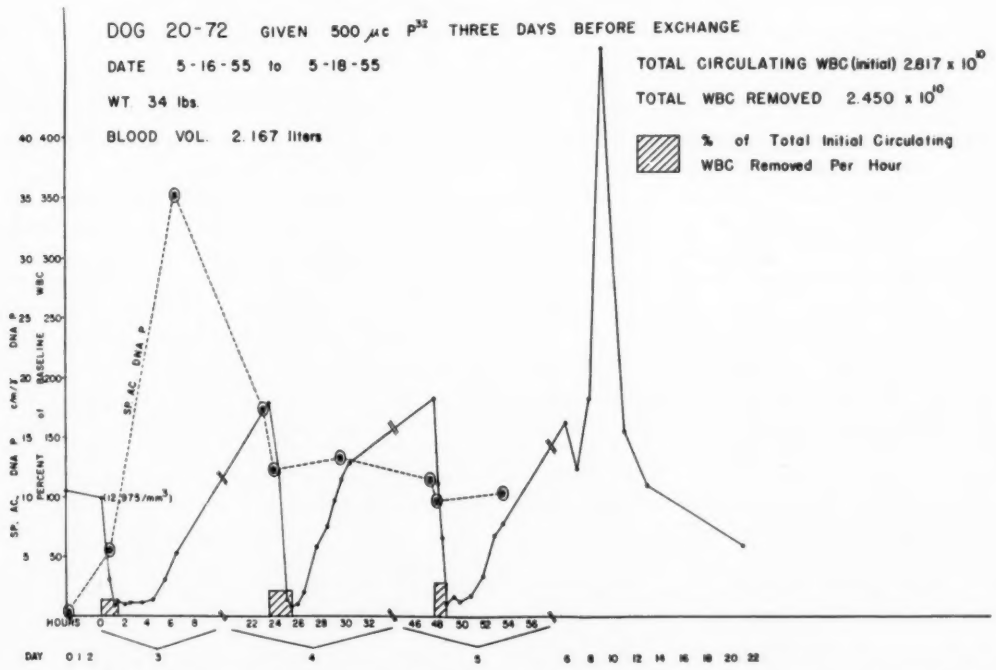
DOG 20-72 GIVEN 500 μc P32 THREE DAYS BEFORE EXCHANGE
DATE 5-16-55 to 5-18-55
WT 34 lbs.
BLOOD VOL. 2.167 liters
TOTAL CIRCULATING WBC (initial) 2.817 x 10^10
TOTAL WBC REMOVED 2.450 x 10^10
% of Total Initial Circulating WBC Removed Per Hour
SP. AC. DNA P
(12,975/mm3)
HOURS
DAY

FIG. 12. THE RESPONSE OF A NORMAL ANIMAL TO REPEATED LEUKOPHERESIS INSTITUTED THREE DAYS AFTER ADMINISTRATION OF 500 μc. P^{32} INTRAVENOUSLY

Note the marked rise in specific activity of DNA P in those cells contributing to the leukocytosis after the first leukopheresis.

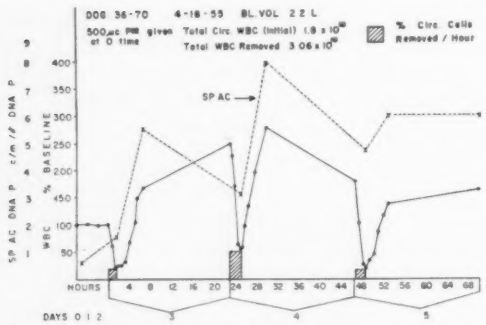
DOG 36-70 4-18-55 BL VOL 2.2 L
500 μc PO4 given at 0 time
Total Circ WBC (initial) 1.8 x 10^10
Total WBC Removed 3.06 x 10^10
% Circ. Cells Removed / Hour
SP AC DNA P c/m / γ DNA P
% BASELINE WBC
SP AC
HOURS
DAYS

FIG. 13. THE RESPONSE OF A NORMAL ANIMAL TO REPEATED LEUKOPHERESIS INSTITUTED THREE DAYS AFTER ADMINISTRATION OF 500 μc. P^{32}

Note the rise in specific activity of DNA P during the period of developing leukocytosis after the first two leukophereses.

of the rise in specific activity observed after the first leukopheresis. Whether or not most of the maximally labeled cells appear after the first leukopheresis or later, as in the case of this dog probably depends upon the timing of the procedure.

Both animals exhibited a fall in specific activity of leukocyte DNA P during leukopheresis. This suggests that leukocytes which are older than the average circulating cell population contribute to the mass of cells removed during leukopheresis. The marked rise in specific activity of DNA P after leukopheresis on one occasion in Dog 20–72 and on two occasions in Dog 36–70 indicate that cells were contributed from the highly labeled marrow pool in much higher percentage than from the relatively unlabeled peripheral tissues.

DISCUSSION

These data indicate the feasibility of experiments on leukocytes employing a method of DNA extraction which we believe to be superior to the Schmidt-Thannhauser principle used by most other workers.

Preliminary results indicate a life span for dog leukocytes considerably shorter than those reported for humans.

The changes in the concentration in the peripheral blood of white cells having DNA labeled with P^{32} support the concept, presented earlier in this paper, that the bulk of cells contributing to the leukocytosis after leukopheresis are released from the marrow and do not come from extravascular and extramedullary areas. Therefore, the response to leukopheresis is believed to represent an accurate measure of the capacity of the marrow to supply the peripheral blood with leukocytes. These findings may be correlated with previous data in irradiated animals (*2*) to indicate that the marrow represents the main, if not the only, reserve supply of leukocytes available for release into the circulation during leukocytosis of the type observed here.

This does not necessarily mean that leukocytosis can occur only by means of accelerated release of cells from the marrow. There is ample evidence indicating the importance of redistribution of leukocytes within the vascular compartment secondary to various hemodynamic alterations, causing fluctuations in peripheral blood white cell concentration (*e.g.*, exercise, epinephrine). Other types of leukocytosis have not been studied by techniques similar to those employed here. It may be that the leukocytosis subsequent to leukopheresis is unique in its mechanisms. Nevertheless, it has revealed an interesting and intricate relationship between the utilization rate of peripheral blood leukocytes and the release of cells from the marrow. This concept is presented in diagrammatic form in Figure 14.

The rate of development and degree of leukocytosis after leukopheresis depend upon the numerical size of the leukocyte reserve. The evidence presented supports the belief that this reserve is primarily within the marrow. Normally the marrow reserve is replaced at such a rate that repeated or continuous leukopheresis as presently applied fails to greatly affect its size. However, damage by irradiation which reduces the replacement of the reserve by growth depresses the response to repeated leukopheresis. The level attained in the blood after leukopheresis becomes progressively less as the size of marrow reserve becomes reduced.

This relationship between the blood level of leukocytes attained after leukopheresis and the size of the marrow leukocyte reserve suggests that the adequacy of the marrow reserve and replacement by growth may be indirectly assessed by this means. Whether other types of stimulation to leukocytosis such as inflammation or infection are associated with a similar relationship between blood and marrow leukocytes remains to be investigated. The data suggest that the release of cells from the marrow is geared to the utilization rate of leuko-

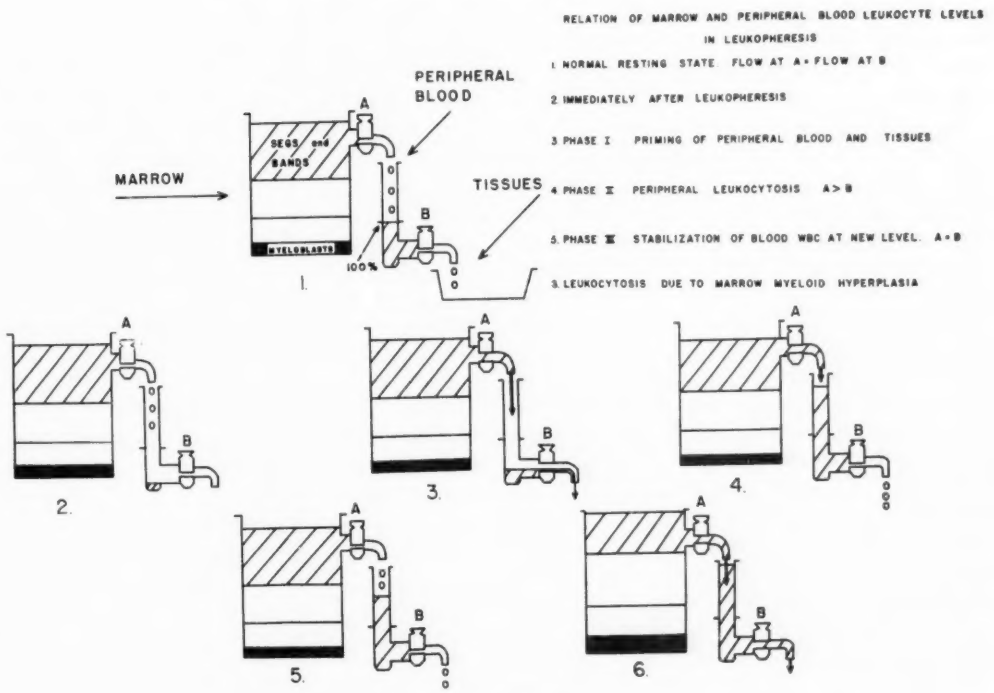

FIG. 14. DIAGRAM

cytes in the periphery. Such utilization may involve actual migration of cells from the blood into the tissues or destruction of leukocytes in the circulation by agglutination, lysis, *etc.* The leukocyte count at a given moment does not reveal whether the rate of utilization is high, low, or normal, and hence cannot yield information as to the rate of entry of leukocytes from the marrow. However, a persistently elevated neutrophile count must reflect an adequate marrow reserve of leukocytes. Conversely, a low neutrophile count, despite obvious stimulation to neutrophilic leukocytosis, must mean a depleted marrow reserve, as in the leukopenia of overwhelming pyogenic infection. Presumably in this latter situation the continued demand for leukocytes in the periphery outstrips the ability of the growth processes to replace those cells released from the marrow reservoir at a rate sufficient to maintain the volume of the marrow reserve. This phenomenon could result from a great demand for leukocytes imposed on a marrow whose reserves were already depleted by previous disease even if the actual rate of cell synthesis were continuing at a normal or accelerated rate. A small percentage of marrow myeloid cells are myeloblasts, and since these represent the ultimate source of mature leukocytes, depletion of the marrow of its contents of mature cells requires time for replacement. Assuming that the level of peripheral leukocytes in the presence of a stimulus to leukocytosis depends upon the size of the marrow reserve, as suggested by our experiments, the blood leukocyte level will remain low despite intense leukopoiesis if the reserve has been greatly reduced.

Looked upon in this way, the large size of the marrow reserve of mature neutrophilic leukocytes in proportion to those circulating becomes an integral factor in leukocyte physiology. The presence of this reserve, described so clearly by Kindred and Yoffey (3, 4), is necessary to prevent the occurrence of marrow depletion by the sudden imposition of a greatly increased rate of peripheral utilization of leukocytes. The formation of new leukocytes is sufficient to balance the demand for leukocytes under resting conditions. However, one can visualize a situation where growth would be inadequate to sustain the need

for myeloid leukocytes if a sudden demand, many times greater than the resting requirement, were imposed before marrow myeloid hyperplasia had taken place. The marrow reserve affords a huge buffer between supply by growth, which is fixed within certain limits, and demand, which may vary widely. The rate and degree of replenishment of the peripheral blood with leukocytes under conditions of increased need is initially geared to the marrow reserve and not to the rate of growth of myeloid tissue. Thus, heavily irradiated animals respond to leukopheresis with a rapid and marked leukocytosis, until the marrow reserves become depleted. Even after marked depletion of the marrow and peripheral leukopenia, the stimulus associated with leukopheresis leads to release of cells from the marrow, but the rise peripherally is very slow and the maximum is very low. The mechanisms involved in the accelerated release of cells from the marrow reserve induced by leukopheresis remain to be investigated. Whether the primary stimulus is a change in the level or concentration of leukocytes in the peripheral blood or the actual increased removal of cells is not entirely clear. However, the results in irradiated dogs (see Figure 5, for example) suggest that the removal of cells represents a stimulus over and above that of leukopenia itself. Thus, in this animal who was leukopenic at the time leukopheresis was instituted (2,070 per mm.^{3}), the marrow responded by releasing cells more rapidly until a new maximum was reached. The mode of transmission of the stimulus to the marrow resulting in accelerated release of leukocytes from the reserve is presumed to be humoral. However, attempts to detect such a humoral agent have been fruitless thus far.

These experiments have provided some background for further study of leukocyte physiology. It is hoped that the information relating leukopoiesis, marrow reserve and peripheral blood leukocytes will aid those interested in derangements in leukopoiesis and leukemia.

SUMMARY

1. The concept is presented that the majority of leukocytes which contribute to an acute leukocytosis are released from areas of storage. Increased production of leukocytes by acceleration of leukopoiesis occurs only after considerable delay, which in the case of the dog subjected to repeated leukopheresis is about three to four days.

2. Evidence is presented that the chief source of mature leukocytes taking part in an acute leukocytosis, such as that observed following leukopheresis, is the marrow reserve. This evidence was obtained by taking advantage of the delay before leukocytes whose DNA becomes labeled with P^{32} during growth move from the marrow into the peripheral blood.

3. Preliminary results of the rate of DNA synthesis in dog neutrophile leukocytes are presented, employing a new method of desoxyribose nucleic acid extraction. These results suggest a shorter life span for this cell in the dog than that reported by others for man.

4. The data are briefly discussed and a concept is formulated which clarifies the relationship of the marrow reserve of leukocytes to those in vascular and extravascular areas.

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THE EFFECT OF NOR-EPINEPHRINE ON THE ELECTROLYTE COMPOSITION OF ARTERIAL SMOOTH MUSCLE[1]

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The changes in the electrolyte composition of arterial wall in renal and desoxycorticosterone induced hypertension (1) have stimulated us to investigate the changes in the electrolytes of arterial smooth muscle that occur when an artery is brought under the influence of nor-epinephrine. The effects of this particular substance are especially important since present evidence indicates that it is the main sympathetic neurohumoral agent affecting the peripheral arterial system. This study was first attempted with arterial rings *in vitro* using the Krebs-Henseleit medium. In this medium the control arteries lost large amounts of potassium, a situation which prompted an *in vivo* approach.

METHODS

All dogs were given a standard dose of Nembutal® for anesthesia. The carotid was cannulated in each dog to provide a continuous recording of arterial blood pressure.

In 12 dogs, the right femoral artery was ligated and quickly excised, stripped of adventitia, blotted free of blood, and placed in a polyethylene weighing bottle for analysis. As soon as the right femoral artery had been excised, an infusion of nor-epinephrine in saline (16 micrograms per cc.) was begun. The infusion lasted 30 minutes, and the rate of the infusion was adjusted to maintain a 100 mm. Hg rise of arterial blood pressure. During the last 5 minutes of the 30-minute infusion, the left femoral artery was quickly excised and treated as described above. Blood samples were drawn at the same time that each femoral artery was excised.

In five control dogs, the same procedure was employed except that there was no nor-epinephrine in the infused saline solution. The saline was given to the control dogs at the same rate as it had been infused into the "nor-epinephrine" dogs.

This experimental plan allows a comparison of one artery obtained before the infusion with the contralateral artery obtained during the last 5 minutes of a 30-minute infusion. In order to determine whether the sequence of obtaining the arteries affected the results, two dogs were subjected to a "reverse" experiment, the nor-epinephrine infusion was started before any artery had been excised. The first femoral artery was obtained during the last 5 minutes of this 30-minute nor-epinephrine infusion. Then the infusion was stopped and the dog was allowed to recover for 45 minutes. After this period of recovery, the other femoral artery was excised and prepared for analysis.

The samples of arterial wall were weighed and then frozen and dried *in vacuo* to a constant weight. After drying they were reweighed to determine the water content as well as the dry weight. The dry arteries were then extracted with known volumes of .75N HNO_3 (2) for 5 days. Aliquots of this extract were analyzed for sodium and potassium by flame photometry. Chloride was analyzed by Lowry's new micromethod (3). All analyses of arterial wall are expressed in terms of 100 gm. of dry solids.

RESULTS

In the group of dogs receiving nor-epinephrine it can be seen from Table I that the average potassium content of the arterial wall before the infusion was 8.1 mEq. per 100 gm. of artery solids. The average potassium content during the last 5 minutes of the infusion was 5.9 mEq. per 100 gm. solids. This average decrease of 2.2 mEq. per 100 gm. solids represents a loss of 27.5 per cent of the initial potassium content of the arteries. Every one of the dogs receiving nor-epinephrine showed a drop in the potassium content of arterial wall during the nor-epinephrine infusion. However, the per cent decrease from pre-infusion levels varied considerably from dog to dog. The smallest drop was 4 per cent, the greatest fall was 59 per cent. In this case, it is remarkable that $\frac{3}{5}$ of the total potassium was lost from the artery during the nor-epinephrine infusion.

Table II shows the potassium content of the femoral artery wall in the control dogs receiving a

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TABLE I

The content of potassium and sodium in artery wall, before and during an infusion of nor-epinephrine (mEq. per 100 gm. of artery solids)

Dog No.	K content before infusion	K content during infusion	Difference	% Change	Na content before infusion	Na content during infusion	Difference
1	7.0	2.9	−4.1	−59	18.7	20.3	+1.6
2	8.4	4.6	−3.8	−45	17.6	22.7	+5.1
3	9.1	5.1	−4.0	−44	18.2	21.1	+2.9
4	9.3	6.0	−3.3	−35	22.4	21.6	−0.8
5	8.3	5.4	−2.9	−35	19.8	22.4	+2.6
6	7.7	5.1	−2.6	−33	21.2	20.3	−0.9
7	8.7	6.7	−2.0	−22	19.6	22.8	+3.2
8	8.0	6.8	−1.2	−16	16.0	18.8	+2.8
9	5.0	4.3	−0.7	−13	21.6	24.4	+2.8
10	8.7	7.6	−1.1	−13	20.1	20.5	+0.4
11	8.9	8.1	−0.8	− 9	23.7	23.8	+0.1
12	8.5	8.2	−0.3	− 4	21.4	21.4	0
Average values	8.1	5.9	−2.2	−28	20.0	21.7	+1.7

saline infusion without nor-epinephrine. Instead of showing a decrease in the potassium content of arterial wall during the infusion, these dogs had an average 17 per cent increase in potassium content during the infusion. Four of the five dogs showed a gain of potassium in the arterial wall during the saline infusion. One dog had a 10 per cent decrease of potassium in the arterial wall during the saline infusion. The dogs receiving nor-epinephrine lost an average of 2.2 mEq. of potassium per 100 gm. of artery solids; the dogs receiving saline averaged a 1.1 mEq. gain in potassium in the arterial wall. This difference is highly significant statistically with a p value of less than .001.

Tables I and II also show the changes in sodium in arterial wall after the various infusions. The dogs receiving nor-epinephrine gained an average of 1.7 mEq. of sodium per 100 gm. of artery solids. This is to be compared with a loss of 2.2 mEq. of potassium in these same arteries. The gain in sodium in arterial wall covers only about ¾ of the loss of potassium. The dogs receiving saline without nor-epinephrine lost on the average 0.4 mEq. of sodium from the arterial wall during the infusion. Here again we have opposite responses in the series of dogs receiving nor-epinephrine and in the control series receiving saline. The series receiving nor-epinephrine shows an average gain in sodium in the arterial wall; the saline series shows a loss of sodium from the artery. The difference between these groups appears to be statistically significant with a p value of .03.

In the series of dogs receiving nor-epinephrine, even though the loss of potassium from the arterial wall was accompanied by an average gain in sodium, the responses in the individual dogs were highly variable. In general the responses could be divided into three types.

The first type represents cases where the decrease of potassium in the artery after nor-epinephrine was fairly well balanced by an increase in sodium. Dogs No. 3 and 5 exemplify this type of response.

TABLE II

The content of potassium and sodium in artery wall before and during an infusion of saline (mEq. per 100 gm. of artery solids)

Dog No.	K content before infusion	K content during infusion	Difference	% Change	Na content before infusion	Na content during infusion	Difference
13	5.6	8.1	+2.5	+45	21.0	20.0	−1.0
14	6.8	8.5	+1.7	+26	19.5	20.6	+1.1
15	8.2	10.0	+1.8	+22	22.2	21.0	−1.2
16	7.1	7.3	+0.2	+ 3	18.9	19.2	+0.3
17	8.6	7.7	−0.9	−10	23.4	22.2	−1.2
Average values	7.2	8.3	+1.1	+17	21.0	20.6	−0.4

TABLE III

"Reverse" nor-epinephrine experiment
(mEq. per 100 gm. of artery solids)

Dog No.	K content during nor-epinephrine infusion	K content 45 min. after infusion	Difference	% Change	Na content during nor-epinephrine infusion	Na content 45 min. after infusion	Difference
18	6.8	8.9	+2.1	+31	22.4	19.8	−2.6
19	8.4	10.5	+2.1	+25	21.9	22.3	+0.4

There are other dogs in whom the rise in sodium in the artery significantly exceeded the decrease in potassium after nor-epinephrine. Dogs No. 2, 7, 8, and 9 are examples of this type.

However, there are six dogs that demonstrate a striking independence in the sodium and potassium changes. Dogs 4 and 6 had a decrease in potassium in the artery after nor-epinephrine and a decrease in sodium as well. Dog 12 had a small drop in potassium with no change in sodium. And dogs No. 1, 10 and 11 had very small gains in sodium in the arterial wall that only compensated for about ⅓ of the loss of potassium. These examples indicate that the changes in potassium content of these artery cells are not merely a passive electrochemical consequence of variations in the activity of a hypothetical "sodium pump mechanism." It appears that in these artery cells, sodium and potassium can vary independently of one another, and each cation probably has a separate active mechanism in the cell membrane regulating its permeability and intracellular accumulation. Nor-epinephrine in certain instances can affect the potassium mechanism in the membrane while not having any influence on the sodium mechanism.

Table III shows a "reverse" nor-epinephrine experiment in two dogs. In dog No. 18 the potassium content of the arterial wall was 6.8 mEq. during the nor-epinephrine infusion and 8.9 mEq. 45 minutes after the infusion had been stopped. In dog No. 19 the potassium was 8.4 mEq. during the infusion and 10.5 mEq. 45 minutes after the infusion. In both of these dogs the potassium content during the nor-epinephrine infusion was lower than during the recovery period when the artery was not under the influence of nor-epinephrine. In one, the potassium loss was replaced by a sodium gain. In the other, there was a loss of both potassium and sodium. The results in this "reverse" experiment agree with those outlined above.

The arteries of the dogs receiving nor-epinephrine lost an average of 7.8 gm. of water per 100 gm. of artery solids during the nor-epinephrine infusion, while the arteries of the control series gained an average of 8.1 gm. of water during the saline infusion. This difference would not be considered statistically significant with a p value of 0.1. However, the arteries lost 0.57 mEq. of total cation (sodium + potassium) per 100 gm. of solids during the nor-epinephrine infusion and gained 0.65 mEq. of total cation during the saline infusion. These changes in total cation content parallel the direction of the changes in water content.

The changes in chloride content of the artery and of the serum were not significantly different in the "nor-epinephrine" group compared to the saline control group. In dogs where the sodium in the artery increased and the chloride did not, there is circumstantial evidence that the sodium was moving into the cells.

The sera of dogs receiving nor-epinephrine lost an average of 1.2 mEq. of sodium and 0.1 mEq. of potassium per liter during the nor-epinephrine infusion. The serum of the control dogs lost an average of 1.2 mEq. of sodium and 0.5 mEq. of potassium per liter during the saline infusion. There was no significant difference between these two groups in regard to the serum changes during the infusions. The changes in the concentrations of sodium and potassium in serum were small during the infusion of nor-epinephrine. They are to be contrasted with the observations of Muirhead, Goth, and Jones who infused much greater quantities of nor-epinephrine and found a considerable decrease in sodium concentration and an increase in potassium concentration in serum during the infusion (4). They postulated that these serum changes might result from sodium entering and potassium leaving cells under the influence of nor-epinephrine. The findings in our study indicate that at least in arteries these shifts do occur. And with their larger doses of nor-epinephrine the ca-

tion shifts in various tissues could have been great enough to lower serum sodium and raise serum potassium.

DISCUSSION

The results indicate that arterial wall invariably lost potassium under the influence of nor-epinephrine, whereas there was a variable gain in sodium to replace the lost potassium. The smooth muscle cells of the arterial wall constitute the bulk of the cells present, and it is likely that the changes in the arterial wall as a whole mainly reflect changes in this smooth muscle. These electrolyte shifts in arterial wall can best be explained by assuming that nor-epinephrine affects the membrane of the smooth muscle cells allowing for a net loss of potassium and a variable gain in sodium.

Nor-epinephrine in fairly high concentration will usually cause a contraction of arterial smooth muscle (5). It is possible that the electrolyte shifts described above play an important part in the chain of chemical events leading to the muscle contraction. The electrolyte shifts could affect the membrane potential of the cell and thus change the tension of the smooth muscle (see below). It is also well known that changes in the concentration of potassium or sodium ions profoundly affect the contractility of actomyosin, presumably by altering the electrical charges on the protein. Such an effect may be occurring in the smooth muscle cells of arteries when nor-epinephrine reduces the content of intracellular potassium.

In nerve, skeletal muscle, and heart muscle, activity or contraction is associated with a loss of intracellular potassium and a gain of intracellular sodium. Our results on arterial smooth muscle would seem to fit in with these observations. However, Daniel, working with rabbit uterine strips, has reported that a contraction of the strip caused by nor-epinephrine produces a definite loss of potassium with a decreased uptake of sodium, while a contraction of the strip caused by either acetylcholine or histamine was associated with no significant change in either potassium or sodium content (6). Hence contraction of uterine smooth muscle, at least, is not invariably associated with a drop in potassium and a gain in sodium.

Recently, Bülbring, working with a longitudinal smooth muscle of the colon, found that the tension of the muscle was always inversely related to the membrane potential, as determined by puncture with micro-electrodes (7). This relationship held during spontaneous activity of the muscle, during the influence of drugs employed to increase tension, and during electrical stimulation of the muscle. If such a relationship were also true for arterial smooth muscle, the membrane potential during physiological contraction might be reduced because of the loss of intracellular potassium. However, this concept remains no more than speculation until some direct evidence can be obtained.

Insofar as dog arteries can be compared with rat arteries, the pattern of electrolyte change associated with nor-epinephrine is different from the pattern of electrolyte change associated with either renal or desoxycorticosterone induced hypertension. With nor-epinephrine the arterial wall loses potassium, while in renal or desoxycorticosterone hypertension the arterial wall gains potassium (1).

SUMMARY

1. Dogs were infused with either nor-epinephrine in saline or saline alone, and samples of arterial wall were obtained before and during each infusion. The samples of arterial wall were analyzed for sodium, potassium, chloride, and water.

2. Under the conditions of these experiments, the femoral artery wall in the dog invariably loses potassium when under the influence of nor-epinephrine. This loss averaged 27 per cent of the total potassium in the artery wall. One dog lost 59 per cent of the total potassium during the nor-epinephrine infusion. Control dogs receiving saline showed an average increase in the potassium content of the arterial wall during the saline infusion.

3. The loss of potassium from the arterial wall during a nor-epinephrine infusion was often accompanied by a gain in sodium. The average gain of sodium in the arterial wall in the group of "nor-epinephrine" dogs was significantly greater than that seen in the control group. However, in half the "nor-epinephrine" dogs, the loss of potassium from the arterial wall was accompanied by little or no increase in sodium. It seemed that the sodium and potassium in artery wall could vary independently under the influence of nor-epinephrine.

4. The decrease of potassium in the arterial wall of dogs after nor-epinephrine is in contrast with the gain in potassium in the arterial wall of rats with renal or desoxycorticosterone hypertension.

5. The loss of potassium from arterial smooth muscle cells may be important in the chain of chemical events associated with a contraction of arterial smooth muscle induced by nor-epinephrine.

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THE PHYSIOLOGIC EXPLANATION OF THE CHANGES IN THE CORONARY CIRCULATION FOLLOWING PROLONGED AORTICO–CORONARY SINUS ANASTOMOSIS [1]

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This is a report of the physiologic changes in the coronary circulation which are precipitated by prolonged periods of arterialization of the coronary sinus.

Initial experiments pertaining to the physiology of the coronary circulation of dogs in which the coronary sinus had been arterialized for four to eight weeks demonstrated a protection to the heart against ventricular fibrillation, following sudden occlusion of the circumflex branch of the left coronary artery. In this group of animals, the myocardium was perfused in retrograde fashion with extraction of oxygen from the blood flowing backward, and the intercoronary collateral flow was increased (1–3).

Subsequently, two series of animals were studied six and twelve months respectively after arterialization of the coronary sinus, to determine whether the retroperfusion of the myocardium, and the protection to the heart against ventricular fibrillation persisted.

METHODS

Two groups of animals, whose weights varied between 15 and 18 kg., were first prepared using the two stage operation developed by Beck (4). At a first stage, through a left-sided approach, an external jugular vein graft was placed between the aorta and the coronary sinus. Approximately one month later, using a right-sided approach, the coronary sinus was partially ligated over a probe to 2 to 3 mm. The first group, Group A, led a normal kennel existence for six months prior to obtaining physiologic data. In the second group, Group B, physiologic data were obtained after twelve months.

At this time, after the animals were anesthetized with intravenous sodium pentobarbital, artificial respiration was maintained with intermittent positive pressure, supplied through a pneophore respirator attached to an endotracheal tube with inflated rubber cuff. The left chest was entered through the fourth intercostal space, the vein graft was inspected, and only those animals in which it was patent were selected for this study. An umbilical tape was placed around the graft to facilitate its occlusion at the appropriate moment during the experiment. A needle-tipped polythene catheter attached to a water manometer was inserted into the coronary sinus, through which its pressure and a blood sample for the determination of oxygen content were obtained. The graft was occluded for two minutes, after which the data were reobtained. The circumflex branch of the left coronary artery was next dissected to its origin, and ligated. An incision was made in the wall of the artery distal to the ligature, through which it was cannulated. A sample of the circumflex coronary arterial blood flowing in retrograde fashion was collected, simultaneous samples being obtained from the aorta and the pulmonary artery, for the determination of oxygen content and saturation data. Back-flow bleeding was recorded quantitatively. The vein graft was then occluded and the same physiological measurements were made again. The peripheral segment of the circumflex coronary artery was allowed to bleed freely for two minutes during which time the retrograde flow volumes were recorded. Blood samples were then collected for determination of oxygen content. The data were collected in this order because in previous experiments, after the vein graft was occluded, the oxygen content of the back-blood emerging from the circumflex coronary artery gradually increased for two minutes before stabilizing.

RESULTS

A. Retrograde bloodflow from the peripheral segment of the ligated and transected circumflex branch of the left coronary artery

1. Group A: (Table I). With the graft open, retrograde blood flow ranged from 3.0 to 18.0 cc. per minute, with an average of 8.3 cc. per minute. Following the occlusion of the graft, the retro-

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grade circumflex arterial blood flow remained unchanged. These values ranged from 3.0 to 17.0 cc. per minute, with an average of 8.7 cc. per minute.

2. Group B: (Table II). With the graft open, the retrograde blood flow ranged from 1.0 to 12.0 cc. per minute, with an average of 6.0 cc. per minute. When the graft was occluded, the retrograde blood flow ranged from 1.0 to 12.0 cc. per minute, with an average of 6.6 cc. per minute.

Essentially, the retrograde blood flows were unchanged by the occlusion of the venous graft.

B. Oxygen content of the retrograde blood collected from the peripheral segment of the transected circumflex artery

1. Group A: (Table I). The values for the oxygen content of this retrograde blood ranged from 10.0 to 17.4 volumes per cent, and averaged 14.6 volumes per cent. Simultaneously drawn samples of aortic blood ranged from 16.4 to 19.1 volumes per cent with an average of 17.7 volumes per cent.

2. Group B: (Table II). The oxygen content of this retrograde blood ranged from 12.9 to 18.7 volumes per cent, and averaged 16.3 volumes per cent. Concomitantly drawn samples of aortic blood ranged from 16.3 to 21.2 volumes per cent, with an average of 19.0 volumes per cent.

C. Peripheral pressure of the distal segment of the transected circumflex coronary artery

1. Group A: These values were not secured in this group.

2. Group B: (Table II). Peripheral coronary arterial pressures ranged from 16 to 40 cm. of water, and averaged 27.5 cm. of water.

When the aortico-coronary sinus vein graft was occluded, the pressures ranged from 16 to 55 cm. of water, with an average of 34.5 cm. of water.

D. Mean coronary sinus pressure and blood oxygen content

1. Group A: (Table I). Initially, the mean coronary sinus pressure ranged from 85 to 100 cm. of water, and averaged 90 cm. of water. The oxygen content of this blood ranged from 14.9 to 19.6 volumes per cent, and averaged 17.4 volumes per cent. After the aortico-coronary sinus vein graft was occluded, coronary sinus pressure rapidly fell to normal. These pressures ranged from 6.0 to 7.5 cm. of water, and averaged 6.4 cm. of water. The blood oxygen content now ranged from 4.8 to 10.3 volumes per cent, with an average of 8.3 volumes per cent.

2. Group B: (Table II). With the graft open, mean coronary sinus pressure ranged from 90 to

TABLE I

Arterialization of the coronary sinus for six months
Retrograde blood flow and oxygen content of blood from the peripheral segment of the ligated and transected left circumflex coronary artery

Dog No.	Graft	Retrograde coronary art. Flow cc./min.	Retrograde coronary art. O_2 cont. Vol. %	Cor. sinus Press. cm. H_2O	Cor. sinus O_2 cont. Vol. %	Aortic blood Cont. Vol. %	Aortic blood Cap. Vol. %	Aortic blood Sat. %	Pul. art. O_2 cont. Vol. %
1	Open	18	10	85	16.7	16.9	16.5	100	11.6
	Closed	17	14.9	6	4.8				
2	Open	9	13.2	90	16.6	16.9	16.9	100	12.4
	Closed	9	18.3	6.5	6.0				
3	Open	4	14.8	90	18.8	19.1	19.3	99	14.8
	Closed	3.8	15.1	6.5	9.8				
4	Open	7.5	17.2	85	19.6	19.0	19.9	95.5	15.5
	Closed	7.5	17.0	6	8.9				
5	Open	3	15.3	90	14.9	16.4	17.8	92	13.5
	Closed	3	17.2	6	10.3				
6	Open	8	17.4	100	17.9	17.8	17.3	100	12.3
	Closed	12	17.2	7.5	9.7				
Av.	Open	8.3	14.6	90	17.4	17.7			13.4
	Closed	8.7	16.6	6.4	8.3				

TABLE II

Arterialization of the coronary sinus for twelve months
Retrograde blood flow and oxygen content of blood from the peripheral segment of the ligated and transected left circumflex coronary artery

Dog No.	Graft	Retrograde coronary artery			Cor. sinus		Aortic blood				Pulm. art.
		Flow *cc./min.*	O_2 cont. *Vol. %*	Press. *cm. H_2O*	Press. *cm. H_2O*	O_2 cont. *Vol. %*	Cont. *Vol. %*	Cap. *Vol. %*	Sat. *%*	Pr. *cm. H_2O*	O_2 cont. *Vol. %*
1	Open	1.2	12.9	26	90	17.7	16.3	16.5	98.8	95	10.3
	Closed	1.2	16.8	55	5.6	10.1					
2	Open	9.0	18.7	27	100	19.2	21.2	19.7	107.1	100	13.3
	Closed	11.0	21.2	39	4.0	9.9					
3	Open	1.0	13.3	16	106	17.6	19.8	18.5	107.0	108	9.0
	Closed	1.0	14.6	16	4.4	1.0					
4	Open	7.8	18.7	40	110	15.7	18.7	19.2	97.3	110	11.0
	Closed	7.5	18.2	37	6.0						
5	Open	12.0	17.8	35	100	18.9	20.6	20.4	100	100	13.7
	Closed	12.0	20.6	33	5.0	11.6					
6	Open	5.0	16.4	21	120	17.7	17.1	18.1	94.5	120	12.3
	Closed	7.0	17.5	27	4.0	10.3					
Av.	Open	6.0	16.3	27.5	104	17.8	19.0				
	Closed	6.6	18.1	34.5	4.8	7.1					11.6

120 cm. of water, with an average of 104 cm. of water. The oxygen content of the arterialized coronary sinus blood ranged from 15.7 to 19.2 volumes per cent, and averaged 17.8 volumes per cent.

When the aortico-coronary sinus vein graft was clamped, the pressure within the coronary sinus rapidly fell to normal, and ranged from 4.0 to 6.0 cm. of water with an average of 4.8 cm. of water. The blood oxygen content now ranged from 1.0 to 11.6 volumes per cent, and averaged 7.1 volumes per cent.

E. Mortality rate following circumflex coronary artery ligation

In order to study all specimens pathologically, an arbitrary survival time of four hours subsequent to the ligation of the circumflex artery was selected as the criterion for a comparative mortality report.

1. Group A: Two dogs survived for the prescribed period of time subsequent to the ligation of the circumflex coronary artery. Four animals died of ventricular fibrillation within twenty minutes of the ligation of the circumflex artery, thus providing a mortality rate of 66 per cent.

2. Group B: Two dogs survived for the prescribed four-hour period prior to sacrifice. Four of the animals died of ventricular fibrillation within one hour of the ligation of the circumflex coronary artery, again providing for a 66 per cent mortality rate. The survivors (animals 2 and 5, Table II), demonstrated the highest rates of retrograde circumflex arterial flow. These flow rates were unchanged following the occlusion of the vein graft, which is probably indicative of the existence of an effective coronary arterial collateral circulation.

F. Pathologic findings

Since the findings that were encountered in the six and twelve-month animals were identical, both groups will be discussed together.

Vein graft: In all animals there was good union between the vein graft and the coronary sinus. The wall of the graft was thickened. There was intimal thickening, due to an increase of collagen and elastic fibers. The media and adventitia were similarly thickened, so that the two coats were indistinguishable. The intima was lined by a single layer of endothelial cells.

Coronary sinus: The changes in the wall of the coronary sinus were more pronounced. The intima was markedly thickened by large numbers of collagen and elastic fibers. This impinged upon the size of the lumen which was decreased up to approximately 50 per cent of normal. The intima was lined by a single layer of endothelial cells. In

three dogs (animals No. 4 Table I, and No. 3 and 4 Table II) the lumen was obliterated by an acellular hyalinizing collagenous mass interpreted as an organized thrombus (Figure 1). The media and adventitia were not distinguishable because of the presence of increased bundles of interlacing collagen and elastic fibers.

Epicardial veins: The most pronounced and advanced changes were noted in the epicardial veins. The walls of these vessels demonstrated a severe degree of intimal fibroelastosis which was extensive enough to narrow the lumen critically so that no more than a slit remained in most cases (Figure 2). In many instances the lumen was replaced by a hyalinizing collagenous mass within which a few capillaries could be found. These were interpreted as being representative of recanalizing organized thrombi. The media and adventitia were not distinguishable because of the extensive fibroelastosis.

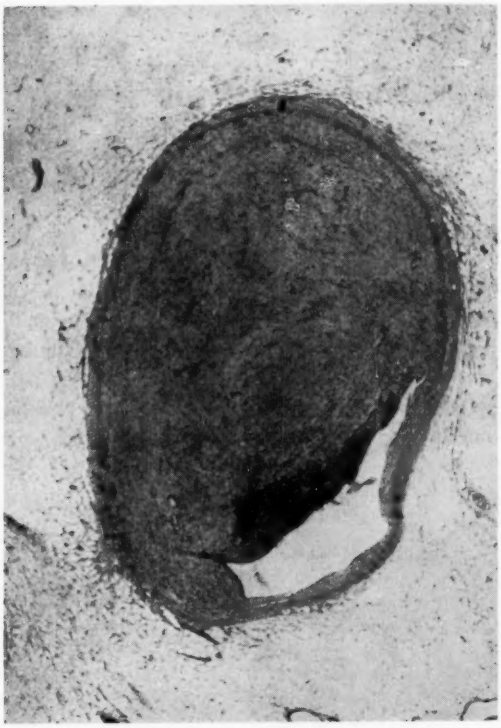

FIG. 1. CORONARY SINUS

The lumen has been almost completely occluded by a large organized thrombus. (64×; H & E.)

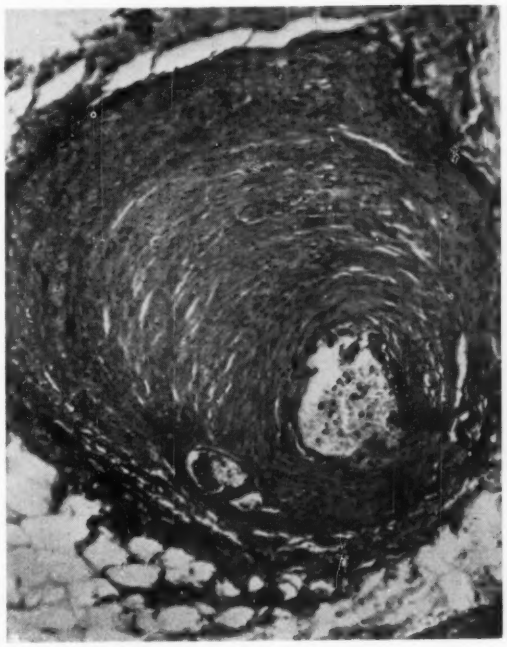

FIG. 2. EPICARDIAL VEIN SHOWING PRONOUNCED INTIMAL PROLIFERATION OF COLLAGEN AND ELASTIC FIBERS WITH EXTREME NARROWING OF LUMEN (120×; H & E)

Coronary arteries: The coronary arteries examined, revealed no changes in their walls.

Myocardium: Focal areas of scarring, seen only microscopically, were found in some cases of both six and twelve-month dogs.

DISCUSSION

Since the results of this experiment are at variance with those in which the coronary sinus was arterialized for short periods of time, it might be valuable to review the preliminary results.

In a group of normal control animals, there was a small quantity of intercoronary collateral flow (1.7 cc. per minute). This blood was invariably arterial in nature, which demonstrated that it had originated from the neighboring coronary arteries. This backflow therefore, represented a quantitative measure of intercoronary arterial collateral flow (1). The mortality rate following circumflex arterial ligation in this group of animals was 90 per cent.

In a second group of animals in which nondescript cardiac procedures had been previously per-

formed, the retrograde blood flow, and oxygen content of this blood, was unchanged from that of the normal control. However, in this group of animals, in which no definitive surgical procedure had been performed to increase the intercoronary collateral circulation, the mortality rate following circumflex coronary artery ligation fell to 66 per cent. *It is this mortality rate that we must subsequently utilize as our baseline when evaluating the effect of any operative procedure specifically designed to increase intercoronary collateral flow.*

In a third series of animals in which the coronary sinus had been arterialized for one to two months, these findings were significantly changed. With the aortico-coronary sinus vein graft open, the retrograde circumflex arterial blood flow averaged 12.2 cc. per minute, a significant increase over that of the expected normal. Moreover, this back-blood differed from that of the control animal in that it was markedly de-oxygenated, the oxygen content being within the range of normal coronary sinus blood. The desaturation of this blood was demonstrative of a retroperfusion of the myocardial capillary bed, with the ability of the myocardium to extract oxygen from this blood. When the vein graft was occluded, the retrograde circumflex coronary arterial blood flow fell to 7.1 cc. per minute, and the oxygen content reverted to normal. The arterial nature of this blood again signified that it had arisen from intercoronary anastomoses, and was suggestive of an increase in the intercoronary arterial anastomotic flow. The mortality rate in this group of animals fell to 20 per cent, which suggested a significant protection to the heart against ventricular fibrillation. This impression has been dispelled by the present series of experiments.

In the animals in which the coronary sinus was arterialized for six months, the findings were significantly changed from those previously described in the one month to two-month group. Now, with the graft functioning, the retrograde flow from the peripheral segment of the circumflex coronary artery averaged 8.3 cc. per minute. This blood was highly oxygenated, the oxygen content approximating that of the simultaneously drawn sample of aortic blood. The arterial nature of this blood indicated that it had originated from neighboring unligated arteries. When the vein graft was occluded, the backflow blood again averaged 8.7 cc. per minute, with no change in its arterial nature. Strikingly then, after six months of aortico-coronary sinus arterialization, neither the quantity nor the oxygen content of the retrograde coronary arterial blood flow was affected by the presence or absence of a functioning aortico-coronary sinus vein graft.

The investigation of the changes in the coronary circulation in animals in which coronary sinus arterialization was maintained for truly prolonged periods of time (twelve months), merely served to reconfirm those of the six-month group of animals. Apparently, the arterialized coronary sinus has completely lost contact with the myocardial capillary bed.

These findings served to confirm those recently reported by Eckstein and his associates (5, 6). These workers were unable to demonstrate retroperfusion of the myocardium after three months of coronary sinus arterialization. It was suggested that blood flowing in retrograde fashion from the peripheral segment of the transected circumflex coronary artery probably originated in the anterior descending artery.

It might be well to review the comparative mortality statistics compiled during this series of physiologic experiments. Ligation of the circumflex branch of the left coronary artery in a group of normal animals resulted in a mortality rate of 90 per cent. This rate fell to 66 per cent in the group in which previous nondescript cardiac procedures had been performed. When the coronary sinus was arterialized for short periods of time, the mortality rate fell to 20 per cent. It was this impressive finding which stimulated the present investigation. These long-term experiments quickly dispelled our previous impressions. In both the six and twelve-month groups of animals there has been no protection afforded to the heart against ventricular fibrillation by the aortico-coronary sinus vein graft. The mortality rate in each group had risen to 66 per cent, which was identical to that found in the group of animals in which only nondescript cardiac procedures had been performed. This experiment has demonstrated that the initial favorable effects produced by arterialization of the coronary sinus regress over a prolonged period of time.

The inability to demonstrate any retrograde

perfusion of the myocardial capillary bed by physiologic means has been further substantiated by the microscopic pathologic findings at the time of postmortem examination. Pathologically, there is a marked thickening of the wall of the coronary sinus, primarily due to intimal proliferation. Moreover, all of the tributaries of the coronary sinus demonstrate a severe degree of intimal proliferation. The lumina of these vessels are so markedly encroached upon by this intimal fibroelastosis, that they may be considered to be completely occluded by this process. In addition, many of the vessels are occluded by organized thrombus, which further serves to isolate the coronary sinus from the myocardial capillary bed. Apparently, prolonged periods of coronary sinus hypertension induce the production of a severe degree of intimal proliferation associated with thrombosis and organization within the small and large veins which empty into it. This intraluminal pathology serves to occlude effectively the vessels, and thereby subsequently obviate the retroperfusion of the myocardial capillary bed.

Since the initial retroperfusion of the myocardial capillary bed is negated after a period of time by the severe pathologic changes induced by the high head of pressure within the coronary sinus and its venous tributaries, it becomes apparent that in the end, the procedure does little more than transiently to increase intercoronary arterial collateral flow. It strikes us that it may be possible to attain a more lasting result by much simpler one stage procedures. We have therefore instituted a series of physiologic experiments to evaluate the simpler procedures designed to produce intra-extracoronary collateral circulation.

SUMMARY AND CONCLUSIONS

1. The retrograde blood flow and oxygen content of the blood from the distal segment of a ligated circumflex artery have been determined in dogs in which the coronary sinus has been arterialized for periods of six and twelve months. These determinations have been performed with the graft open and closed.

2. With the graft open, coronary sinus pressure is within the range of that of the systemic circulation, and the coronary sinus blood is of high oxygen content.

3. When the graft is occluded, the coronary sinus pressure and blood oxygen content revert to those of normal coronary sinus levels.

4. With the graft open, retrograde circumflex coronary arterial blood flow is small in quantity but is greater than that expected in the normal control animal. This blood is arterial in nature, approximating the oxygen content of aortic blood, and is probably due to an increase in the intercoronary arterial collateral circulation.

5. Occlusion of the aortico-coronary sinus vein graft does not significantly alter these findings. This indicates that after a prolonged period of time, the aortico-coronary sinus vein graft no longer contributes to or affects the inter-arterial collateral circulation, and is therefore dispensable.

6. The mortality rate following circumflex coronary arterial ligation in both groups of animals is 66 per cent, which is unchanged from that in a series of animals in which nondescript cardiac procedures had been performed.

7. Retroperfusion of the myocardial capillary bed can no longer be demonstrated in this group of animals. Moreover, we were unable to demonstrate any protective effect upon the myocardium against ventricular fibrillation following coronary arterial occlusion in either group of animals.

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STUDIES OF THE ANTIDIURESIS OF QUIET STANDING: OBSERVATIONS UPON ELECTROLYTE AND ACID–BASE EXCRETION DURING SULFATE DIURESIS[1]

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It is well established that when blood is pooled in the limbs, either by the use of congesting tourniquets or by standing erect and motionless, there is an immediate and marked decrease in urinary flow and in the excretion of sodium and chloride (1, 2). This is usually accompanied by a slight diminution in glomerular filtration rate and by little or no change in the excretion of potassium and ammonium or in urinary pH (2–4). The mechanisms by which sodium excretion is diminished remain unclear, although considerable evidence suggests that the rates of urinary excretion of sodium and other strong electrolytes are interrelated through a process of ionic exchange in the renal tubules (5). It was thought that these relationships might be illuminated by measuring changes in the excretion of K^+ and H^+, when reabsorption of Na^+ was acutely increased but total cation reabsorption limited, by assuming the upright posture during the course of a sodium sulfate diuresis.

METHODS

Experiments were carried out on normal young men, in the morning after a light breakfast. An isotonic solution of sodium sulfate (248 mEq. per L., 296 mOsm. per L.)[2] was infused intravenously at 4 to 5 cc. per min. over a period of six hours in all experiments. Four kinds of experiments were performed. In *Group I* (control) the subject remained supine for the duration of the procedure. In *Group II* he stood upright and motionless during the fifth hour of the infusion. In *Groups III and IV* the latter procedure was repeated while an effort was made to alter renal tubular secretion with a carbonic anhydrase inhibitor (Diamox®) or an organic mercurial (Thiomerin®). On three occasions (*Group III*) subjects ingested 500 mg. of Diamox® at the start of the experiment and 250 mg. at the beginning of the fourth hour. In three subjects (*Group IV*) 1 cc. of Thiomerin® was incorporated in each liter of the infusion and 2 cc. of Thiomerin® were injected intramuscularly at the beginning of the second hour.

Samples of arterialized venous blood and of urine, voided under mineral oil, were collected every half-hour or hour. Serum and urine were analyzed for endogenous creatinine (7), sodium and potassium (8), chloride (9, 10) and carbon dioxide (11). Sulfate was determined by a turbidimetric technique (12) in the earlier experiments and later by a modified colorimetric method using benzidine (13, 14). pH of blood and urine was determined with a Cambridge pH meter. Urine was analyzed for ammonia (15) and in most cases for titratable acidity.

RESULTS

I. *Continuous infusion of sodium sulfate in the supine position (Table I, Figure 1)*

The rates of excretion of sodium and sulfate increased rapidly during the first three hours but continued to rise only very slowly during the fourth, fifth and sixth hours, as equilibrium between the intake and output of sulfate was approached. During the last three hours the clearance of sulfate progressively approached the clearance of endogenous creatinine. Although the excretion of potassium invariably increased from the first to the second hour, this was not true of ammonium, and during the final three hours the excretion of K^+ and NH_4^+ was fairly constant. The excretion of chloride at first increased slightly but then progressively declined to very low levels. The normal diurnal variation in urinary pH was unaltered. Urinary flow and the clearance of endogenous creatinine changed little during the six hours of infusion.

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[2] Seventeen and one-half gm. of Na_2SO_4 (anhydrous) were dissolved in 1,000 cc. of distilled water. This solution has the same freezing point as a solution of NaCl containing 148 mM. per kg., although it contains 248 mEq. per L. of Na_2SO_4. The osmotic coefficient of Na_2SO_4 in this range of concentration is 0.74 (6).

TABLE I

Urinary constituents during the fourth, fifth and sixth hours of a control infusion of isotonic sodium sulfate in supine subjects

Subject	Hours after start of infusion	Posture	Urine							
			Flow	Na^+	K^+	NH_4^+	pH	Cl^-	$SO_4^=$	C_{cr}
			cc./min.	*μEq./min.*	*μEq./min.*	*μEq./min.*		*μEq./min.*	*μEq./min.*	*cc./min.*
F. E.	4	Supine	3.61	940	123	24	6.70	67	1,162	128
	5	Supine	3.28	948	122	27	6.19	29	1,185	123
	6	Supine	3.32	1,050	113	26	6.20	34	1,280	130
E. L.	4	Supine	2.02	728	150	42	5.48	32	895	140
	5	Supine	2.02	780	134	42	5.08	21	921	146
	6	Supine	2.05	830	105	38	5.22	18	950	142
M. R.	4	Supine	4.88	895	85	40	5.08	51	1,010	132
	5	Supine	4.92	1,025	74	36	5.42	71	1,120	139
	6	Supine	5.00	1,180	55	35	6.00	105	1,232	140
C. K.	4	Supine	2.62	861	111	23	6.63	58	955	121
	5	Supine	2.21	818	101	28	5.58	22	944	115
	6	Supine	2.42	957	88	34	5.00	15	1,093	126
D. W.	4	Supine	3.92	1,160	121	28	6.65	98	1,304	120
	5	Supine	4.13	1,165	103	25	6.50	76	1,258	113
	6	Supine	3.94	1,230	96	23	6.65	58	1,342	110
Mean	4		3.41	917	118	31	6.11	61	1,065	128
	5		3.31	947	107	31	5.76	44	1,085	127
	6		3.35	1,049	91	31	5.61	46	1,179	127

II. *Effects of motionless standing during the fifth hour of a sodium sulfate infusion* (*Table II, Figure 2*)

Standing caused a decrease in the excretion of sodium in each of five subjects and produced a urine almost free of chloride, while sulfate excretion remained essentially unchanged. The excretion of both potassium and ammonium, on the other hand, increased in the erect posture and usually diminished when the subject lay down again. There was an abrupt decrease in urinary pH, and values as low as 4.27 (uncorrected for the difference between room and body temperature) were recorded during or shortly after the period of standing. These striking increases in the acidity of the urine were not preceded or accompanied by changes in the pH of arterialized blood or in serum bicarbonate. Endogenous creatinine clearances increased slightly in one subject and fell an average of 11 cc. per min. in four others. Sustained by the continued excretion of sulfate, the flow of urine diminished only slightly during the stimulus of quiet standing.

Experienced subjects reported that while standing erect during sulfate infusions symptoms of faintness, giddiness and nausea appeared earlier and more regularly than during prolonged quiet standing without sulfate. These symptoms were even more pronounced in the experiments of *Groups III and IV*, and always disappeared promptly when the subject lay down.

III. *Effect of a carbonic anhydrase inhibitor* (*Table III*)

In three men to whom Diamox® had been administered, quiet standing during the fifth hour

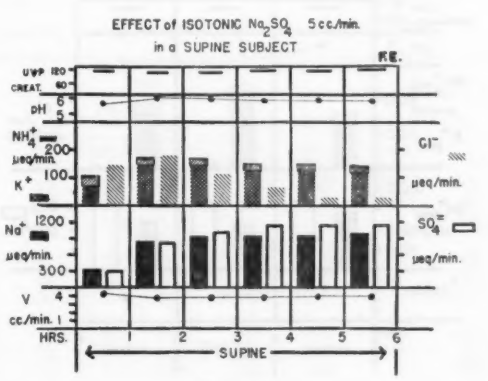

FIGURE 1

TABLE II

Effects of the erect posture upon electrolyte excretion during an infusion of sodium sulfate

Subject	Hours after start of infusion	Posture	Urine							
			Flow	Na^+	K^+	NH_4^+	pH	Cl^-	$SO_4^=$	C_w
			cc./min.	*μEq./min.*	*μEq./min.*	*μEq./min.*		*μEq./min.*	*μEq./min.*	*cc./min.*
F. E.	4	Supine	3.40	843	137	31	5.62	15	1,023	118
	5	Standing	2.47	741	228	38	4.33	4	1,025	112
	6	Supine	2.12	666	180	30	4.83	3	911	101
E. L.	4	Supine	2.41	773	145	44	6.38	69	874	143
	5	Standing	2.04	745	176	56	4.67	18	966	146
	6	Supine	2.09	764	162	49	5.10	9	987	149
M. R.	4	Supine	2.66	1,039	60	40	4.80	33	1,160	125
	5	Standing	2.02	853	84	50	4.27	11	1,023	99
	6	Supine	2.71	989	101	54	4.32	4	1,188	120
C. K.	4	Supine	3.71	1,130	117	18	6.96	151	1,223	147
	5	Standing	2.30	896	127	39	4.85	25	1,131	144
	6	Supine	2.27	994	88	38	4.82	9	1,223	144
D. W.	4	Supine	3.20	1,025	152	27	6.44	33	1,242	111
	5	Standing	2.52	923	188	37	4.85	17	1,250	100
	6	Supine	2.67	1,066	169	33	4.99	15	1,326	112
Mean	4	Supine	3.08	962	122	32	6.04	60	1,104	129
	5	Standing	2.27	832	161	44	4.59	13	1,079	121
	6	Supine	2.37	896	140	41	4.81	8	1,127	125

of a sulfate infusion was accompanied by a decreased excretion of sodium, chloride, bicarbonate and endogenous creatinine. In contrast to the preceding experiments, however, *urinary pH and the excretion of ammonium did not change appreciably.* The excretion of potassium, already at a high level, did not increase further when the subject stood up.

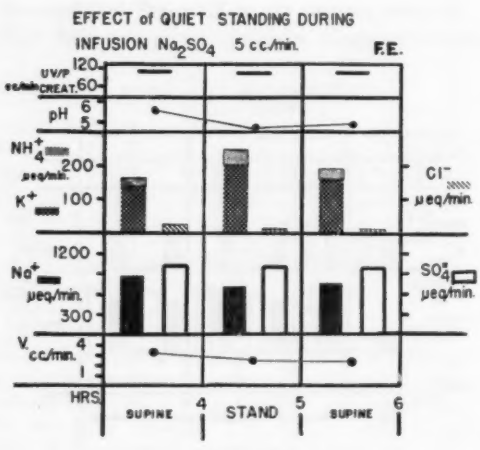

FIGURE 2

IV. *Effect of an organic mercurial (Table IV)*

In three subjects receiving Thiomerin®, the retention of sodium which accompanied motionless standing *was not associated with an increased excretion of potassium.* However, as in *Group II,* urinary pH became more acid and the excretion of ammonium increased.

Infusion of sodium sulfate, which normally diminishes the excretion of chloride (*Group I*), did not prevent the chloruretic effect of mercury in these experiments. Chloride excretion was therefore high throughout the procedure, although it fell in each case during the period of quiet standing.

DISCUSSION

The present experiments demonstrate once again that changing from the supine to the standing posture provides a specific stimulus to the renal tubular reabsorption of the sodium ion. The consequent retention of sodium is not accompanied by an increase in the rates of excretion of potassium and ammonium when quiet standing is superimposed upon a diuresis induced by water ingestion (16, 17), infusion of physiological saline (4, 17),

TABLE III

Modification by Diamox® of the response to quiet standing during an infusion of sodium sulfate

Subject	Hours after start of infusion	Posture	Urine								
			Flow	Na^+	K^+	NH_4^+	pH	HCO_3^-	Cl^-	$SO_4^=$	C_{in}
			cc./min.	*μEq./min.*	*μEq./min.*	*μEq./min.*		*μEq./min.*	*μEq./min.*	*μEq./min.*	*cc./min.*
E. L.	4	Supine	6.04	1,165	211	17.1	7.57	360	78	1,010	142
	5	Standing	3.97	1,020	201	17.5	7.50	210	17	1,010	135
	6	Supine	3.98	1,163	184	17.6	7.41	183	20	1,120	148
F. E.	4	Supine	6.20	1,416	224	10.8	7.54	381	125	1,080	110
	5	Standing	2.50	740	227	10.8	7.07	62	20	850	89
	6	Supine	3.29	1,055	200	10.0	7.20	95	10	1,130	99
M. E.	4	Supine	5.50	1,190	221	6.6	7.63	355	134	852	122
	4½	Standing	3.63	970	220	5.2	7.56	262	78	855	122
	5	Standing	2.11	680	174	3.6	7.44	110	33	655	87
	6	Supine	3.33	1,230	211	6.3	7.43	180	30	1,110	148

or infusions of mannitol, sodium bicarbonate or sodium phosphate (4). The fact that urinary outputs of potassium, ammonium and hydrogen ions were simultaneously increased when sodium retention was provoked during the infusion of a non-reabsorbable anion suggests alterations in tubular activity since this cannot be explained by the hypothesis of a lowered rate of glomerular filtration in the presence of unchanged tubular reabsorption. Furthermore, changes in glomerular filtration as indicated by clearances of endogenous creatinine were frequently small or absent in these experiments. However, the data do not permit a final decision as to whether changes in tubular activity might not possibly have been a by-product of lowered glomerular filtration, as Lauson and Thompson demonstrated in dogs whose renal artery was compressed (18).

In view of current theories of urinary acidification which postulate an inverse relationship between the rates of secretion of potassium and hydrogen ions (19), it is of interest that increases in the production of ammonium and marked falls in urinary pH were promoted acutely by quiet standing with a simultaneous increase in the excretion of potassium. Moreover, abrupt and intense acidification of the urine was produced without systemic acidosis. Analogous findings have been reported by Lauson and Thompson in dogs (18), by Schwartz, Jenson, and Relman in human subjects depleted of sodium or given adrenal steroids (20), and by other investigators who have given large loads of non-reabsorbable anions to patients with a tendency to retain sodium (21, 22).

The increased excretion of potassium and acid during the period of quiet standing must have been

TABLE IV

Modification by mercury of the response to quiet standing during an infusion of sodium sulfate

Subject	Hours after start of infusion	Posture	Urine									
			Flow	Na^+	K^+	NH_4^+	Titr. acidity	pH	Cl^-	$SO_4^=$	HCO_3^-	C_{in}
			cc./min.	*μEq./min.*	*μEq./min.*	*μEq./min.*	*μEq./min.*		*μEq./min.*	*μEq./min.*	*μEq./min.*	*cc./min.*
C. K.	4	Supine	4.22	1,238	60	22	1.6	6.68	365	865	33	92
	5	Standing	3.84	1,080	64	32	9.5	5.40	305	864	0	77
	6	Supine	10.0	2,215	61	28	2.4	6.23	1,030	1,125	34	93
E. L.	4	Supine	6.50	2,240	137	41	1.0	6.75	1,422	985		92
	5	Standing	6.40	1,315	132	48	12.5	5.94	530	950		91
	6	Supine	6.40	1,974	178	38	1.0	6.70	947	1,150		112
F. E.	4	Supine	5.52	1,140	143	19	3.0	6.93	249	780	54	120
	5	Standing	2.02	520	140	32	10.0	4.72	31	475	0	67
	6	Supine	8.35	1,540	163	30	8.8	5.66	745	980	0	108

secondary either to diminished tubular reabsorption or to increased tubular secretion of these substances. The results of the studies with Diamox® and mercury might be considered to favor the latter view. The dose of Diamox® administered would be expected to inhibit tubular secretion of H^+ and to accelerate to a maximum the secretion of K^+ (19). Under these circumstances the stimulus of quiet standing was ineffective in changing the rates of excretion of either of these ions, although sodium was retained. Administration of mercury, which inhibits renal tubular secretion of K^+ in dogs (23), blocked the increase in K^+ excretion which usually accompanied motionless standing, but did not prevent a rise in ammonium excretion and fall in urinary pH. The possibility cannot be excluded that increased amounts of chloride and bicarbonate in the tubular urine contributed to and modified these results by permitting the reabsorption of Na^+ with a reabsorbable anion. The data are compatible, however, with the theory that the renal retention of sodium produced acutely by quiet standing is caused in part by increased tubular reabsorption of sodium from urine through a mechanism of ionic exchange with tubular cells for both potassium and hydrogen ions. This process, not ordinarily apparent, is unmasked during a sulfate diuresis by the obligatory excretion in the urine of a large number of negative sulfate ions which require neutralization.

SUMMARY

1. Quiet standing during the course of a sodium sulfate diuresis produced the following changes:

a. An abrupt decrease in the renal excretion of sodium and chloride.

b. Little change in urine flow, creatinine clearance, or the excretion of sulfate.

c. An increase in the excretion of potassium and ammonium and a fall in urinary pH.

2. Administration of Diamox® prior to quiet standing prevented the increase in excretion of potassium and acid during quiet standing.

3. Administration of Thiomerin® prior to quiet standing blocked the increase in potassium excretion but did not prevent the formation of a more acid urine with an increase in the excretion of ammonium.

4. The results suggest that under these circumstances both potassium and hydrogen ions may participate simultaneously in the reabsorption of sodium by the renal tubules through an active process of ionic exchange.

ACKNOWLEDGMENT

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STUDIES ON MYOCARDIAL METABOLISM. V. THE EFFECTS OF LANATOSIDE-C ON THE METABOLISM OF THE HUMAN HEART [1]

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Since 1785 when Withering undertook the first systemic study of the effects of digitalis, a large amount of data has been accumulated describing its pharmacological effects. Despite intensive study, the underlying mechanism responsible for the beneficial results in patients with heart failure remains obscure. Although digitalis in small doses may affect the heart rate through its vagal effect, it is agreed that the predominant action of this drug is on the heart muscle directly (1). In addition, the cardiac glycosides also influence the ionic gradient between the muscle cell and the surrounding medium (2–8). Its effect on oxygen consumption and foodstuff utilization of the heart has been repeatedly investigated in the heart-lung preparation, in tissue slices and in tissue homogenates (9). The reader is here referred to the review article of Wollenberger (10). However, the abundance of conflicting results suggests that cardiac glycosides may exhibit different metabolic defects dependent on the experimental condition.

It appears likely that one limiting factor in elucidating the mechanism of action of the glycoside has been the lack of means for direct study of the metabolic responses of the heart beating in a more physiological environment. By the technique of coronary sinus intubation, a direct study of patients treated with therapeutic doses of a rapid acting cardiac glycoside is possible. Simultaneous sampling of arterial and coronary venous blood, together with measurement of the coronary flow, permits the determination of the net myocardial gain or loss of foodstuffs, gases or other metabolites (11–13). While providing no information regarding intermediary reactions, data obtained by this method should aid in elucidating the metabolic pathways within the heart muscle; thus reactions determined by more precise *in vitro* methods could be re-evaluated in the light of data obtained on the human heart *in vivo*.

The various cardiac glycosides differ in time of onset and persistence of action. Lanatoside-C, the preparation used in this study, is a purified derivative of digitalis lanata which can be given intravenously. The onset of action depends on the dosage used; with average digitalizing doses of 1.2 to 1.6 mgm., the so-called "vagal effect" usually appears within 15 to 20 minutes, reaching a maximum in about 40 minutes. Weisberger and Feil (14), for example, reported their results in treating 17 cases of resistant paroxysmal auricular tachycardia. Sixteen cases reverted to normal sinus rhythm within 40 minutes, with an average response of 17.6 minutes. Barrow (15), in a series of 26 patients, found the average time for reversion to be 19 minutes. Nicholson (16) administered 1.6 mgm. of Cedilanid® to 22 subjects with auricular fibrillation whose rates were stabilized at 120 beats per minute. In all patients there was a drop of from 15 to 80 beats within 15 minutes, with an average decrease in heart rate of 24 beats. Stead, Warren, and Brannon (17) administered 1.6 mgm. of lanatoside-C intravenously to 22 patients with congestive failure. The first effect noticed was a fall in venous pressure which began in 5 to 10 minutes and continued for 30 to 60 minutes. Cardiac output, measured 60 to 70 minutes after the injection, showed an average rise of 1.6 liters per minute. This effect was noted regardless of the cardiac rhythm or the initial stroke volume.

The present paper reports on the action of lanatoside-C on the myocardial metabolism of glucose, pyruvate, lactate, amino acids, ketones and

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fatty acids. A subsequent report will deal with the effect of this glycoside on the sodium and potassium balance of the human heart.

PROCEDURE

Twelve undigitalized adult patients were selected for the study (Table I). Seven of these had heart disease with evidence of left ventricular hypertrophy but had no symptoms of congestive failure other than decreased exercise tolerance. Five of these seven were hypertensive (J. B. J., G. G., E. E., W. H., and M. T.), one had rheumatic heart disease (D. T.) and one had aortic insufficiency due to syphilis (F. M.). Congestive heart failure was present in four patients. Heart failure in these individuals resulted from rheumatic valvular disease (L. W.), hypertension (W. B.) and from congenital malformation of the heart (E. F.). In the fourth patient the cause of the failure was unknown (J. R.). One of the subjects in the series had no evidence of heart disease (J. W.).

All patients were studied in the post adsorptive state. In three of the subjects (W. B., E. F., and J. R.) with congestive failure preliminary treatment with bed rest, diuretics and salt restriction was necessary before the studies could be performed. Small doses of merperidine or pentobarbital were given on the morning of the study to those individuals who seemed unduly apprehensive. The majority received no premedication.

Cardiac catheterization was performed in the usual manner (12, 13). If diagnostic procedures were indicated, these were completed first. Cardiac output was measured by the method of Fick (18), the mixed venous sample being obtained from the most distal cardiac chamber entered. Expired air was collected in a Douglas bag or Tissot spirometer. Oxygen and carbon dioxide in expired air was determined by the method of Scholander (19). The coronary sinus was intubated according to previously described methods (20). Simultaneous blood samples were collected from the coronary sinus and right femoral artery for determinations of oxygen, carbon dioxide, glucose, pyruvate, lactate, fatty acids, amino acids and ketones. In most cases duplicate samples were drawn and the results of the two analyses averaged. Coronary flow was measured 30 to 40 minutes following the ingestion of the glycoside. Coronary flow was not determined prior to the injection of lanatoside-C, since this would have unduly prolonged the procedure; also it had been previously found that digitalis in therapeutic doses does not influence the rate of coronary blood flow (21).

Lanatoside-C was administered intravenously in doses of .02 to .03 mgm. per kil body weight, and coronary blood flow was measured using the nitrous oxide desaturation method (22, 23). Thirty to forty minutes later, or as soon as definite electrocardiographic changes appeared (slowing of heart rate, depression of ST segment or premature ventricular contractions), blood samples for metabolic determinations were again obtained from the femoral artery and coronary sinus.

Oxygen and carbon dioxide contents of blood were determined by the manometric method of Van Slyke and Neill (24). Blood glucose was measured by the method of Hagedorn and Jensen, using Somogyi's method to prepare the filtrates (25, 26). Pyruvate was determined according to the method of Friedemann and Haugen (27), using a tricloracetic acid filtrate, and lactate by the method of Barker and Summerson (28). For the determination of fatty acids the method of Man and Gildea was used (29). This is essentially a modification of the procedure of Stoddard and Drury's volumetric analysis (30). Amino acids were estimated according to the method of Albanese and Irby (31) and ketones by a modification of the micromethod of Greenberg and Lester (32).

From the analysis of the blood samples the myocardial extraction (coronary arteriovenous difference) of oxygen, carbon dioxide and individual metabolites was determined. The myocardial usage of 100 grams of left ventricle was calculated as the product of the myocardial extraction times the coronary flow. The oxygen extraction ratio was determined by multiplying the myocardial extraction by the oxygen equivalent of the particular metabolite divided by the myocardial oxygen extraction. This ratio represents the percentage of oxygen extracted by the heart which could be accounted for by complete catabolism of foodstuff to carbon dioxide and water. Because of the temporary storage of metabolites and their conversion within the metabolic pool, the sum of the individual oxygen extraction ratios at any one moment need not total 100 per cent.

The percentage myocardial extraction represents the percentage of the arterial concentration of a metabolite which is removed from coronary blood during its passage through heart muscle; it is calculated by the formula: $\frac{A-V}{A} \times 100$, where A and V represent the respective arterial and coronary venous concentrations of the metabolites. It has previously been shown that extraction of a substrate by the human heart varies in proportion to the arterial concentration (11). Since the percentage myocardial extraction takes into consideration the fluctuations in arterial level, this calculation provides a more accurate means of comparing changes in uptake of carbohydrates, fats and proteins by the myocardium than does the arteriovenous difference alone.

Evidence of Cedilanid® effect was obtained from comparisons of electrocardiograms taken before and at intervals of five to ten minutes following the injection of the glycoside. The earliest and most consistent change observed was slowing of the ventricular rate, which was present in nine subjects by the end of thirty minutes and was most marked in the two patients with auricular fibrillation (L. W. and E. F.). In seven subjects with normal sinus rhythm the average decrease amounted to 10 beats per minute. Other electrocardiographic changes included depression of the S-T segment in three patients, flattening of the T-waves in five patients, and the onset of frequent ventricular extrasystoles after digitalis administration in one individual. No electrocardiographic alterations were present in one patient, and the tracings of one subject were lost. However, the development of

TABLE I

Analysis of data obtained before and after intravenous injection of Cedilanid®

No.	Patient	Age	Sex	Diagnosis		Cardiac index *L./M²/m.*	R.Q. of heart	Coronary flow *(cc./100 gm./min.)*	Arterial levels and myocardial extractions											
									Δ Oxygen *vol. %*	Arterial glucose *mgm. %*	Δ Glucose	Arterial pyruvate *mgm. %*	Δ Pyruvate	Arterial lactate *mgm. %*	Δ Lactate	Arterial fatty acids *mEq./100 cc.*	Δ Fatty acids	Arterial amino acids *mgm. N/100 cc.*	Δ Amino acids	Arterial ketones *mgm. %*	Δ Ketones
1	J. J.	29	M	HCVD	Before	3.58	.73	52	11.1	85.2	1.9	0.484	0.019	7.20	1.59	0.960	0.015	4.54	0.11	0.863	−0.039
					After		1.11		12.3	83.0	5.4	0.511	−0.131	7.20	3.46	0.975	0.035	4.47	0.05	0.922	0.157
					Change		.38		1.2	−2.2	3.5	0.027	−0.150	0.0	1.87	0.015	0.020	−0.07	−0.06	0.059	0.196
2	G. G.	48	F	HCVD	Before	3.17	.77	71	12.6	89.1	4.7	0.525	0.070	7.94	2.01	1.350	0.0	3.76	0.06	0.874	0.092
					After		.75		13.4	91.3	5.4	0.455	0.095	7.29	3.36	1.368	0.048	3.80	0.0	0.898	0.139
					Change		−.02		0.8	2.2	0.7	−0.070	0.025	−0.65	1.35	0.018	0.048	0.04	−0.06	0.024	0.047
3	E. E.	36	F	HCVD, anemia	Before	5.99	.76	112	10.2	95.6	2.1	0.788	0.105	7.67	0.52	1.580	0.015	4.65	0.10	—	—
					After		—		—	92.7	3.9	0.885	0.123	7.47	0.51	1.550	0.005	4.67	0.02	—	—
					Change		—		—	−2.9	1.8	0.097	0.018	−0.20	−0.01	−0.030	−0.010	0.02	−0.08	—	—
4	H. W.	47	F	HCVD, anemia	Before	3.05	.57	113	7.9	96.1	5.6	0.412	0.054	4.44	0.73	1.210	0.020	3.72	0.02	1.379	0.311
					After		.61		7.5	88.9	3.2	0.331	0.053	4.88	0.45	1.230	0.040	3.70	0.0	1.201	0.290
					Change		.04		−0.4	−7.2	−2.4	−0.081	−0.001	0.44	−0.28	0.020	0.020	−0.02	−0.02	−0.178	−0.021
5	M. T.	60	F	HCVD	Before	3.40	.78	61	9.8	—	—	0.434	0.081	6.97	1.99	1.095	0.025	4.46	0.21	2.650	0.980
					After		.60		10.3	—	—	0.402	0.080	7.19	1.47	1.070	0.0	4.25	0.0	1.734	0.25
					Change		−.18		0.5	—	—	−0.032	−0.001	0.22	−0.52	−0.025	−0.025	−0.21	−0.21	−0.916	−0.730
6	D. T.	37	M	Rheumatic heart disease	Before	2.15	.82	98	14.9	82.5	4.1	0.448	0.161	7.64	3.56	1.580	0.005	4.44	0.0	0.849	0.040
					After		.70		13.2	76.1	5.2	0.448	0.144	8.59	3.23	1.550	0.005	4.50	0.0	0.849	0.243
					Change		−.12		−1.7	−6.4	1.1	0.0	−0.017	0.95	−0.33	−0.030	0.0	0.06	0.0	0.0	0.203
7	F. M.	48	M	Syphilitic aortitis; aortic insufficiency	Before	3.89	.72	—	9.3	86.7	1.5	0.473	0.000	6.83	0.69	—	—	—	—	4.51	1.23
					After		.59		10.0	83.9	1.6	0.538	0.033	6.30	0.31	—	—	—	—	4.64	1.39
					Change		−.13		0.7	−2.8	0.1	0.065	0.033	0.53	0.38	—	—	—	—	0.13	0.16
8	L. W.	31	F	Rheumatic heart disease, congestive failure	Before	2.05	.69	—	13.8	94.9	1.2	0.405	0.067	8.09	0.39	1.235	0.025	4.44	0.09	0.843	0.422
					After		—		—	89.7	1.8	0.288	0.076	6.80	1.42	1.185	0.030	4.40	0.0	0.843	0.338
					Change		—		—	−5.2	0.6	−0.117	0.009	1.29	1.03	−0.050	0.005	−0.04	−0.09	0.0	−0.084
9	W. B.	58	M	HCVD, anemia, congestive failure	Before	5.53	.62	104	10.2	103.0	1.5	0.397	0.138	9.54	3.60	1.223	0.015	3.59	0.19	2.44	0.710
					After		.88		10.3	110.2	2.3	0.439	0.137	8.59	3.17	1.087	0.012	3.30	0.14	2.59	0.750
					Change		.26		0.1	7.2	0.8	0.042	−0.001	−0.95	−0.43	−0.136	−0.003	−0.29	−0.05	0.15	0.040
10	E. F.	58	M	Congenital heart disease, congestive failure, ventricular septal defect	Before	2.45	.87	54	10.3	90.2	7.9	0.421	0.114	7.09	3.00	0.765	0.006	4.65	0.20	0.884	0.17
					After		.83		10.8	87.8	7.0	0.397	0.057	7.01	2.94	0.750	0.0	4.53	0.19	0.925	0.21
					Change		−.04		0.5	−2.4	−0.9	−0.024	−0.057	−0.08	−0.06	−0.015	−0.006	−0.12	−0.01	0.041	0.04
11	J. R.	42	M	Heart failure with left ventricular hypertrophy, cause unknown	Before	2.63	.98	71	12.7	88.1	4.9	0.422	0.076	6.67	2.57	0.995	0.010	—	—	1.890	1.03
					After		.69		13.3	85.1	6.1	0.406	0.152	6.85	2.46	0.915	0.020	—	—	1.320	0.52
					Change		−.29		0.6	−3.0	1.2	−0.016	0.076	0.18	−0.11	−0.080	0.010	—	—	−0.570	−0.51
12	J. W.	68	M	Normal	Before	3.20	.64	66	12.0	90.0	0.8	0.388	0.008	6.09	0.67	1.410	0.015	4.35	0	2.750	1.060
					After		.58		13.4	92.0	1.0	0.445	0.057	6.91	2.35	—	—	—	—	1.750	0.400
					Change		−.06		1.4	2.0	0.2	0.057	0.049	0.82	1.68	—	—	—	—	−1.000	−0.660
					Range		.38 to −.29		1.4 to −1.7	7.2 to −7.2	3.5 to −2.4	0.097 to −0.117	0.076 to −0.150	0.95 to −1.29	1.87 to −0.52	0.020 to −0.136	0.048 to −0.025	0.06 to −0.29	0 to −0.21	0.150 to −1.000	0.203 to −0.730
					Mean		−.016		.37	−1.88	.609	.0043	−.0014	.0025	.3808	−.0313	.0059	−.07	−.0644	−.2054	−.1199
					t		.25		1.32	1.48	1.36	.23	.09	.01	1.52	1.78	.92	1.78	3.08	1.62	1.15
					p		>.80		>.20	>.20	>.20	>.80	>.90	>.90	>.20	>.10	>.30	>.10	<.02*	>.10	>.20

p—probability.
* Significant difference.

TABLE I—*Continued*

No.	Patient	Age	Sex	Diagnosis		Myocardial usage (*per 100 gm. L.V./min.*)							Oxygen extraction ratios (*per cent*)							
						Oxygen *cc.*	Glucose *mgm.*	Pyruvate *mgm.*	Lactate *mgm.*	Fatty acids *mEq.*	Amino acids *mgm. N*	Ketones *mgm.*	Glucose	Pyruvate	Lactate	Fatty acids	Amino acids	Ketones	Total carbohydrates	Total noncarbohydrates
1	J. J.	29	M	HCVD	Before	5.8	0.99	0.01	0.83	0.008	0.057	0.02	12.8	0	10.7	77.1	4.6	0	23.5	81.7
					After	6.4	2.81	−0.07	1.80	0.018	0.026	0.08	32.9	0.11	21.1	162.2	1.9	1.30	54.1	165.4
					Change	0.6	1.82	−0.08	0.97	0.010	−0.031	0.10	20.1	0.11	10.4	85.1	−2.7	1.30	30.6	83.7
2	G. G.	48	F	HCVD	Before	8.9	3.34	0.05	1.49	0.0	0.043	0.07	28.0	0.36	11.9	0	2.2	0.74	40.3	2.9
					After	9.5	3.83	0.07	2.39	0.034	0.0	0.10	30.2	0.45	18.8	204.2	0	1.06	49.5	205.3
					Change	0.6	0.49	0.02	0.90	0.034	−0.043	0.03	2.2	0.09	6.9	204.2	−2.2	0.32	9.2	202.4
3	E. E.	36	F	HCVD, anemia	Before	11.4	2.35	0.12	0.58	0.017	0.112	—	15.4	0.66	3.8	83.8	4.6	—	19.9	—
					After	—	4.37	0.14	0.57	0.006	0.022	—	—	—	—	—	—	—	—	—
					Change	—	2.02	0.02	−0.01	−0.011	−0.090	—	—	—	—	—	—	—	—	—
4	H. W.	47	F	HCVD, anemia	Before	8.9	6.33	0.06	0.88	0.023	0.023	0.35	53.6	0.43	6.9	144.3	1.2	4.01	60.9	149.5
					After	8.5	3.62	0.06	0.51	0.045	0.0	0.33	32.0	0.45	4.5	304.0	0	3.94	37.0	307.9
					Change	−0.4	−2.71	0.0	−0.37	0.022	−0.023	−0.02	−21.6	0.02	−2.4	159.7	−1.2	−0.07	−23.9	159.9
5	M. T.	60	F	HCVD	Before	6.0	—	0.05	1.21	0.015	0.128	0.60	—	0.52	15.2	145.0	10.0	10.20	—	165.2
					After	6.3	—	0.05	0.90	0.0	0.0	0.15	—	0.50	10.7	0	0	2.48	—	2.5
					Change	0.3	—	0.0	−0.31	−0.015	−0.128	−0.45	—	−0.02	−4.5	−145.0	−10.0	−7.72	—	−162.7
6	D. T.	37	M	Rheumatic heart disease	Before	14.6	4.02	0.16	3.49	0.005	0.0	0.04	20.6	0.69	17.9	19.1	0	0.27	39.2	19.4
					After	12.9	5.09	0.14	3.16	0.005	0.0	0.24	29.6	0.70	18.3	21.6	0	1.88	48.6	23.5
					Change	−1.7	1.07	−0.02	−0.33	0.0	0.0	0.20	9.0	0.01	0.4	2.5	0	1.61	9.4	4.1
7	F. M.	48	M	Syphilitic aortitis; aortic insufficiency	Before	—	—	—	—	—	—	—	12.1	0	5.5	—	—	13.5	17.6	—
					After	—	—	—	—	—	—	—	12.0	0.21	2.3	—	—	14.2	14.5	—
					Change	—	—	—	—	—	—	—	−0.1	0.21	−3.2	—	—	0.7	−3.1	—
8	L. W.	31	F	Rheumatic heart disease, congestive failure	Before	—	—	—	—	—	—	—	6.5	0.31	2.1	103.2	3.1	3.12	8.9	109.4
					After	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
					Change	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	W. B.	58	M	HCVD, anemia, congestive failure	Before	10.6	1.56	0.14	3.74	0.016	0.198	0.74	11.0	0.87	26.5	84.8	8.7	7.10	38.4	100.6
					After	10.7	2.39	0.14	3.30	0.013	0.146	0.78	16.7	0.85	23.5	66.4	6.4	7.42	41.1	80.2
					Change	0.1	0.83	0.0	−0.44	−0.003	−0.052	0.04	5.7	−0.02	−3.0	−18.4	−2.3	0.32	2.7	−20.4
10	E. F.	58	M	Congenital heart disease, congestive failure, ventricular septal defect	Before	5.6	4.27	0.06	1.62	0.003	0.109	0.09	57.5	0.71	21.8	33.2	9.08	1.68	80.0	44.0
					After	5.8	3.78	0.03	1.59	0.0	0.103	0.11	48.6	0.34	20.4	0	8.24	1.98	69.3	10.2
					Change	0.2	−0.49	−0.03	−0.03	−0.03	−0.006	0.02	−8.9	−0.37	−1.4	−33.2	−0.84	0.30	−10.7	−33.8
11	J. R.	42	M	Heart failure with left ventricular hypertrophy, cause unknown	Before	9.0	3.48	0.054	1.82	0.007	—	0.73	28.9	0.38	15.2	44.9	—	8.28	44.5	—
					After	9.5	4.33	0.108	1.75	0.014	—	0.37	34.4	0.73	13.9	85.9	—	3.99	49.0	—
					Change	0.5	0.85	0.054	−0.07	0.007	—	−0.36	5.5	0.35	−1.3	41.0	—	−4.29	4.5	—
12	J. W.	68	M	Normal	Before	7.9	0.53	0.01	0.44	0.099	0	0.70	5.0	0.04	4.2	71.3	0	9.02	9.2	80.5
					After	8.8	0.66	0.04	1.55	—	—	0.26	5.6	0.27	13.2	—	—	3.00	19.1	—
					Change	0.9	0.13	0.03	1.11	—	—	−0.44	0.6	0.23	9.0	—	—	−6.02	9.9	—
					Range	0.9 to −1.7	2.02 to −2.71	.054 to −0.08	1.11 to −0.44	0.034 to −0.015	0 to −.128	.20 to −0.45	20.1 to −21.6	0.35 to −0.37	10.4 to −4.5	204.2 to −145.0	0 to −10.0	1.61 to −7.72	30.6 to −23.9	202.4 to −162.7
					Mean	.0122	.4455	−.001	.142	.0045	−.0471	−.0977	1.3889	.061	1.09	36.987	−2.7429	−1.3550	3.1778	3.3314
					t	.42	.95	.09	.74	.87	3.11	1.18	.36	.99	.62	.93	2.17	1.28	.63	.07
					p	>.60	>.30	>.90	>.40	>.40	<.02*	>.40	>.70	>.30	>.50	>.30	>.05	>.20	>.50	>.90

p—probability.
* Significant difference.

nausea and vomiting forty minutes after the administration of Cedilanid® (ten minutes after blood samples were taken) probably constitutes adequate evidence of drug effect in this person.

The individual observations before and after Cedilanid® administration are shown in Table I. Differences observed after injection of the glycoside were subjected to statistical analysis, each patient serving as his own control. Changes were considered significant only if there was less than one chance in twenty that the effect could have been due to chance alone ($p < .05$).

RESULTS

No significant changes in the respiratory quotient of the heart could be attributed to the action of Cedilanid®. Of the ten observations available before and after treatment, the respiratory quotient decreased in seven and increased in three (Table I). The myocardial oxygen extraction for the group showed a mean increment of 0.37 volumes per cent, increasing in eight subjects and decreasing in two. The difference was not statistically significant ($p > .20$). Since digitalis causes no significant alteration in coronary flow, it can be assumed that myocardial oxygen usage likewise remained unchanged (21).

Observations of the arterial concentration and coronary arteriovenous glucose difference were available in eleven subjects. The arterial glucose concentration decreased in eight and increased in three patients, while the myocardial extraction decreased in two subjects and increased in nine. For the group as a whole, there was an average fall of 1.9 mgm. per 100 cc. in the arterial level of glucose and an average rise of 0.6 mgm. per 100 cc. in its myocardial extraction. Neither of these changes was statistically significant. Because of the lower arterial level, the percentage glucose extraction by the heart showed a more conspicuous gain, increasing from 3.7 per cent to 4.5 per cent. Although the p value was $> .10$, slightly above the level accepted as significant in this study, it can be seen in Figure 1 that the percentage extraction increased in all subjects except the two who initially had the highest percentages of glucose extraction (W. H. and E. F.).

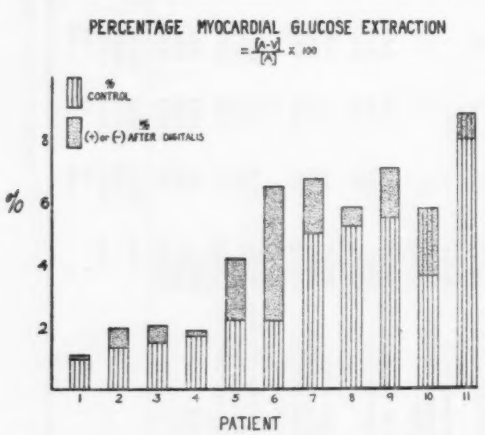

FIG. 1. ILLUSTRATES THE PERCENTAGE MYOCARDIAL GLUCOSE EXTRACTION IN THE ELEVEN PATIENTS STUDIED

This ratio increased in all but two patients (10 and 11); these had the highest initial percentage of glucose extraction.

Myocardial pyruvate extraction showed small fluctuations in both directions, but no consistent changes occurred which could be ascribed to the drug. There was a slight gain from 16.4 per cent to 19.2 per cent in the mean percentage pyruvate extraction. However, in four individual observations this ratio declined.

Alterations in arterial lactate level and myocardial lactate extraction were similarly inconsistent. As in the case of glucose and pyruvate the mean percentage extraction advanced slightly, rising from a control level of 24 per cent to 29 per cent after the administration of Cedilanid®.

Data of fatty acid metabolism were available in ten subjects. The arterial concentration of fatty acids decreased in seven and increased in three individuals with a mean increase of .031 mEq. per 100 cc. ($p > .10$). Changes in fatty acid extraction and percentage extraction were more variable and did not approach statistical significance. The average percentage extraction by the heart increased from 1.2 per cent to 1.7 per cent. Despite the small percentage of available fatty acids extracted, their importance as a potential source of energy to the heart muscle is demonstrated by their high oxygen extraction ratios. In several instances the quantity of fatty acids removed from the coronary circulation could account for more than 100 per cent of the simultaneous oxygen extraction if it is assumed that they are completely metabolized to carbon dioxide and water. Similar findings have been described in previous publications (11, 13).

In eight of nine patients on whom data were available the amino acid extraction by the heart

declined. In one subject myocardial extraction of amino acids was zero before and after injection of Cedilanid®, and in four others the extraction fell to zero. It should be noted, however, that the arterial amino acid concentration also declined in six of the patients, so that the decrease in percentage extraction was not as marked as was the coronary arteriovenous difference. The mean percentage extraction before and after therapy was 2.5 per cent and 1.1 per cent, respectively. The myocardial metabolism of ketones was not altered by digitalis.

DISCUSSION

The mass of conflicting data in the literature makes it likely that the difference in metabolic effects of digitalis on heart muscle may be the result of various concentrations of drug employed and the differences in the kind of substrates and the techniques used in the studies (10, 33, 34). However, even with identical techniques and concentrations of drugs the results have been contradictory. For example, various observers have noted that Ouabain increased the oxygen consumption of cardiac muscle slices respiring in the Warburg apparatus, while others have reported a decreased oxygen uptake with the same concentration of glycosides (10, 33). Using human auricular appendages obtained at operation, Burdette (35) found that the addition of lanatoside-C tripled the rate of respiration. David (36) on the other hand, found that after an initial increase Ouabain caused a diminution in the respiration of the isolated frog's auricle.

Studies on the heart-lung preparation have yielded equally variable results. Evans (37) reported that while digitalis increased cardiac output and efficiency, the oxygen consumption of the heart diminished. Peters and Visscher (9) found that digitalis increases both oxygen consumption and efficiency of the perfused heart. However, the increase in efficiency on a percentile basis was four to five times as great as the increase in myocardial oxygen consumption (9).

The bulk of evidence suggests that in the various types of experimental "failure" digitalis increases the work performance of the heart out of proportion to the changes in oxygen consumption, thereby increasing myocardial efficiency. In patients with congestive failure cardiac glycosides increase cardiac output and efficiency without causing significant changes in oxygen consumption (21).

The results of the present study demonstrate that digitalis in therapeutic dosage produces only minimal changes in the extraction of foodstuffs by the heart. Since there is some rise in the mean extraction percentage of pyruvate and lactate, the lower amino acid extraction after digitalis may be due to the "protein sparing" effect of carbohydrates.

The negative effect of Cedilanid® on carbohydrate metabolism in the intact human heart contrasts with results of Wollenberger (34). This investigator found, in studying the action of Ouabain on the respiration of heart slices in the Warburg apparatus, that the glycoside accelerates the oxidation of glucose and lactate. This action accounts for the simultaneous increase in respiratory activity, with the acceleration of glucose oxidation exceeding, in proportion, that of the oxygen uptake. The results reported in this paper demonstrate only a slight elevation in percentage glucose extraction without any change in myocardial oxygen utilization. Since myocardial lactate utilization remains unchanged, a digitalis-induced shift from a glycolytic to a largely respiratory metabolism of carbohydrates as suggested by Wollenberger is unlikely in the human heart under these conditions.

The finding that the cardiac glycoside produces no significant changes in myocardial oxygen consumption or in total foodstuff utilization suggests that the improvement in work capacity of the failing heart induced by these glycosides must be the result of their action on energy liberation, or more specifically, on the contractile proteins of failing heart muscle directly. Digitalis can accelerate the spiraling of myosin threads (38). Mallov and Robb (39) reported that actomyosin threads prepared from beef hearts showed increased spiraling and shortening when digitoxin was added. In a later report the same authors, using protein surface films, found that Ouabain increased the work performance of actomyosin prepared in this manner (40). Digitalis also influences the polymerization of actin (41). Furthermore, digitoxin does not combine with sarcosomes of heart muscle, in which the enzymes concerned with energy production are concentrated (42). Additional evidence that cardiac glycosides do not influence energy

production is available from the findings of Wollenberger (43) that Ouabain or Digoxin in therapeutic dosages causes no change in the concentration of high-energy phosphate carriers and phosphocreatine. Similar conclusions can be drawn from the work of Rothlin, Taeschler, and Cerletti (44). These investigators induced heart failure in the heart-lung preparation with dinitrophenol, a drug which increased myocardial oxygen consumption. Lanatoside-C restored the initial work performance of the heart without further influencing myocardial oxygen consumption.

SUMMARY

The effects of therapeutic doses of Cedilanid® on the metabolism of glucose, pyruvate, lactate, amino acids, fatty acids and ketone bodies of the human heart have been investigated. The studies were carried out on patients using intubation of the coronary sinus.

Only minimal changes in the extraction of metabolites by the heart were observed. The findings suggest that the improvement in work capacity of the failing heart induced by the glycoside is the result of its action on energy liberation.

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A RAPID PLETHYSMOGRAPHIC METHOD FOR MEASURING THORACIC GAS VOLUME: A COMPARISON WITH A NITROGEN WASHOUT METHOD FOR MEASURING FUNCTIONAL RESIDUAL CAPACITY IN NORMAL SUBJECTS [1]

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In general, two principles have been employed in the measurement of the volume of gas in the lungs. The dilution techniques, using either a closed (rebreathing) or open (non-rebreathing) system are the most commonly used; the physical methods, based on Boyle's law relating pressure and volume, require special equipment and have been used relatively infrequently. This report presents a rapid, practical physical method for measuring lung volumes plethysmographically; it has been found to give reproducible values which, in healthy subjects, do not differ significantly from values obtained by the dilution method of Darling, Cournand, and Richards (1).

Two types of physical (pneumatometric) techniques have been described previously, both based on Boyle's law. One of these is the decompression method used by Hitchcock, Edelmann, Shelden, and Whitehorn (2), Willmon and Behnke (3), and DeJours and Rahn (4); it has been used in experimental animals (5, 6) as well as in normal man. In the decompression method, the pressure about the body and in the trachea and lungs is raised equally above atmospheric so as to compress the gas inside the lungs and outside the body. The total number of molecules is thereby increased in all parts of the lung in free communication with an open airway. The pressures in the chamber and in the trachea are then released simultaneously. During return to normal atmospheric pressure, the amount of gas which flows out of the trachea is measured; this is the amount of gas which had been added to the lung gas by the previous increment of pressure. The pulmonary gas volume at atmospheric pressure can then be calculated by application of Boyle's law. Since the method measures the gas volume which flows in and out of the trachea during compression and decompression, it measures the volume of lung gas which is in communication with the trachea.

The other type, based on voluntary compression or decompression of lung gas, was described in principle by Pflüger in 1882 (7). It requires measurements of changes in alveolar gas pressure and volume while the subject makes voluntary respiratory efforts against a closed airway. It gives values for the volume of gas in the lungs and thorax, whether in free communication with the airway or not. Despite attempts by several investigators to devise an accurate and practical method, it has not been considered to be reliable for clinical or physiological use (8). The development of new methods for measuring and recording pressure changes led us to reinvestigate this technique.

METHOD

The method used is based on Boyle's law that the volume of a gas varies in inverse proportion to the pressure to which it is subjected. At the beginning of the test, the subject has an unknown volume of gas in his chest; at either end-inspiration or end-expiration, when there is no air flow, the pressure of this alveolar gas is known to be atmospheric. If his airway is then occluded so that no pulmonary gas can escape, and he compresses the pulmonary gas by a voluntary expiratory effort, the pulmonary gas now has a new pressure and volume. The change in pulmonary gas pressure can be measured readily during airway occlusion since mouth pressure equals alveolar pressure in a closed system. The change in pulmonary gas volume can be measured by utilizing an air-tight body plethysmograph and a sensitive electrical capacitance manometer. From knowl-

[1] This investigation was supported in part by a research grant, H-406 from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

edge of the original pulmonary gas pressure and of the change in pulmonary gas pressure and volume, the original volume of gas in the chest can be calculated; the derivation of the thoracic gas volume equation is given below.

Details of the method

The subject enters and sits in an airtight chamber, the body plethysmograph (Figure 1), which has the dimensions of a telephone booth (9). The door is closed and the subject breathes the ambient air during the five-minute period of the test. Since the volume of the box is about 600 liters, the oxygen concentration would drop from 20.93 per cent to only about 20.7 per cent and the carbon dioxide concentration would rise from 0.04 per cent to only 0.25 per cent during the test period.

The pressure of the air in the plethysmograph, relative to atmospheric pressure, is measured by a sensitive Lilly capacitance manometer and is recorded on the horizontal axis of a cathode ray oscillograph (CRO).[2] The amplification is such that 0.025 cm. H_2O pressure change produces a horizontal deflection of one inch on the screen; this corresponds to a volume change of 15 ml. in the 600-liter box. The system is calibrated directly in terms of a volume change, with the subject in the plethysmograph, by the rapid introduction and withdrawal of 30 ml. of air.

Immediately after the door is closed, the pressure in the box rises rapidly because air in the box is warmed and humidified by the subject. During this initial period, the box is vented periodically to the room outside by means of a solenoid operated valve until the pressure drift with the vent closed is so slight that it does not interfere with the measurement of the thoracic gas volume; this requires about two minutes. The subject then applies a nose clip and breathes through the mouthpiece-shutter system (*S*, Figure 1) similar to that described by Mead and Whittenberger (10). The solenoid-controlled shutter can be closed by the operator on the outside when it is desired to obstruct the flow of air into and out of the subject's lungs. At the end of a normal expiration, as judged by observing box pressure, the shutter is closed by the operator and the subject continues to make one or more respiratory efforts.

When the subject makes breathing motions against the closed shutter, he alternately compresses and decompresses the air within his chest by the action of his chest muscles. The pressure changes within the lung can be measured by means of a second electrical capacitance manometer which records pressure at the mouth on the pulmonary side of the closed shutter (*P*, Figure 1). In such a closed system, pressure at the mouth is considered to be the same as alveolar pressure if the subject's glottis remains open and his cheeks are held fairly rigid. Mouth pressure is recorded on the vertical axis of the cathode ray oscillograph (*O*, Figure 1). The sensitivity of this system is such that a 5 cm. H_2O pressure change produces a vertical deflection of one inch on the CRO. With this recording system, during occlusion of the airway and continued breathing efforts, changes in pulmonary pressure are graphed continuously against changes in thoracic gas volume on the CRO and appear as a slanting line which can either be traced (Oscillotracer[3]) or photographed. The slope of this line is $\frac{\Delta P}{\Delta V}$.

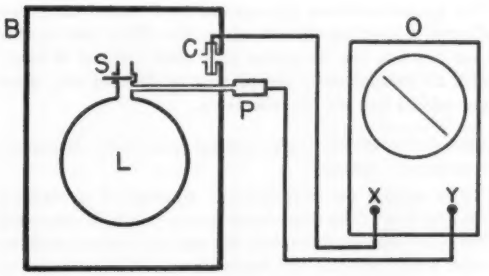

FIG. 1. DIAGRAM OF APPARATUS

S = shutter which occludes airway; L = lung; C = capacitance manometer to record pressure changes in the plethysmograph (which are proportional to change in body volume); P = capacitance manometer to record pressure changes in the mouth (which are equal to alveolar pressure when there is no airflow); O = cathode ray oscillograph with X and Y axes.

The subject may be requested to make one of two types of breathing efforts when the airway is obstructed: 1) The operator closes the shutter at the end of a normal expiration and the subject continues with the next inspiration until he senses that his breathing is obstructed. 2) The subject is asked to make respiratory efforts as though panting; the panting type of breathing is usually preferred because the pressure and volume changes are retraced very quickly several times on the screen and a representative slope is obtained. Furthermore, with the panting type of breathing, the intrathoracic pressure changes are smaller during the test period, and not unidirectional. In either case, after a rest period of a few seconds of unobstructed breathing, the shutter can be closed again and the measurement repeated; 4 to 5 determinations can be made easily in a minute and the average of the slopes is used in the calculations.

Unsatisfactory tracings result if the subject closes his glottis, since mouth pressure no longer equals alveolar pressure; in this case a horizontal line is produced on the CRO because box pressure changes while mouth pressure remains the same. Unsatisfactory tracings also result if the subject moves his cheeks with the breathing motions; in this case, the change in mouth pressure is less than alveolar pressure and lags in time because of a pressure fall across the trachea owing to air flow. This type of breathing is easy to detect visually; it can also be noted on the record which now consists of a loop instead of a straight line.

[2] Dumont CRO, type 304-A, which has satisfactory D. C. gain, persistent screen with flat tube face, bright line grid, and direct coupling of X and Y input.

[3] R. A. Waters, Inc., Waltham, Mass.

The pressure-volume changes can be recorded on a two channel ink-writer instead of on the CRO, but in this case the data can be plotted only after the test is over; thus an unsatisfactory record may be detected only after the subject has left the laboratory.

Derivation of the thoracic gas volume equation for the plethysmographic method

The record for measurement consists of a slanting straight line. The slope between two points is measured, one point representing initial pressure and volume and the other a final pressure and final volume. If P_1 is the initial pressure, $P_1 + \Delta P$ is the final pressure, V_1 the initial volume, and $V_1 + \Delta V$ the final volume, then from Boyle's law:

$$P_1V_1 = (P_1 + \Delta P)(V_1 + \Delta V) \qquad (1)$$

Simplifying:

$$P_1 \Delta V + V_1 \Delta P + \Delta V \Delta P = 0 \qquad (2)$$

Solving:

$$V_1 = -\frac{\Delta V}{\Delta P}(P_1 + \Delta P) \qquad (3)$$

If ΔP is small compared to P_1, then $P_1 + \Delta P \rightarrow P_1$[4] and

$$V_1 = \frac{-P_1(\Delta V)}{(\Delta P)} \qquad (4)$$

In this equation, V_1 is the initial thoracic gas volume or V_{TG}, and $P_1 = 970$ cm. H_2O, which is atmospheric pressure minus water vapor pressure.[5] Therefore, disregarding the sign:

$$V_{TG} = 970\,\frac{(\Delta V)}{(\Delta P)} \qquad (5)$$

The slope of the record (λV_{TG}) is the change in mouth pressure per unit change of box volume, or $\left(\frac{\Delta P}{\Delta V}\right)$. Then:

$$V_{TG} = \frac{970}{\lambda V_{TG}} \qquad (6)$$

When the calibration factors are included, the final equation becomes:

$$V_{TG} = \frac{970 \times \text{box calibration}}{\lambda V_{TG} \times \text{pressure calibration}}$$

In practice the box calibration is usually 15 ml. per inch horizontal deflection and the pressure calibration is usually 5 cm. H_2O per inch vertical deflection so that λV_{TG} would be 1.0 when the V_{TG} is 2,910 ml. which is the most accurate range at these settings.

RESULTS

1. *Accuracy of plethysmographic method*

Initial experiments were carried out to determine the accuracy with which known volumes of gas could be measured by the plethysmographic method. The apparatus was modified so that a subject seated inside the plethysmograph could increase or decrease his pulmonary gas volume by a known volume by inspiring from or expiring into a spirometer outside the plethysmograph. Thoracic gas volumes were measured first after a maximal expiration and then after stepwise inspiration from the spirometer. Then the experiment was repeated after the subject made a maximal inspiration and stepwise expirations into the spirometer. The results obtained in two subjects are as follows: The amount of air that was inspired from or expired into the spirometer on the outside ranged from 0.50 to 5.12 liters. The plethysmograph method measured the increment or decrement in the lung volume. In a total of 35 measurements, the difference of the means between spirometer volume and plethysmographic lung volume was 0.04 liter (S.D. ± 0.17 liter; S. E. ± 0.03 liter).

2. *Reproducibility of plethysmographic method*

The variation among repeated measurements in the same individual was checked by doing multiple determinations of the thoracic gas volume in each of three subjects. Subjects 1 and 2 were trained laboratory workers; subject 3 was an untrained patient with pulmonary arteriosclerosis. The number of measurements were 12, 12 and 23 in these three cases yielding mean values of 3.77, 2.87 and 4.31 liters; two standard deviations from the mean value represented an error of 4.8 per cent, 5.6 per cent, and 3.7 per cent of the mean value for the thoracic volume. Because of the ease in making repeated determinations, all values for thoracic gas volume in the remainder of this paper are the average of 3 to 5 individual determinations performed within a period of 1 to 3 minutes.

3. *Comparison of plethysmographic and dilution method in normal subjects*

Measurements of resting end-expiratory thoracic gas volume (plethysmographic method) and of FRC. (open circuit method of Darling, Cournand, and Richards [1]) were made in each of nine sitting subjects; measurements were usually made within an hour of one another. The subjects included three women and six men who ranged in age from 20 to 43 years. The results are shown in Table I. The mean values were 2.97 liters by

[4] As ΔP approaches zero, $P_1 + \Delta P$ approaches P_1, ΔP and ΔV each approach zero, but the ratio $\frac{\Delta V}{\Delta P}$ becomes the slope $\frac{dV}{dP}$, and the equation becomes $V_1 = \frac{-P_1\,dV}{dP}$.

[5] Water vapor saturated, when compressed, condenses to a liquid, so that its partial pressure is constant, and equal to 47 mm. Hg at 37° C.

TABLE I

Comparison of resting end-expiratory thoracic gas volume measured by plethysmographic method and FRC measured by open circuit method in normal subjects

Subject	Age (yrs.) and sex	Height *inches*	Weight *pounds*	Thoracic gas volume (Plethysmographic) *liters*	FRC (Open circuit) *liters*	Plethysmographic minus Open circuit *liters*
PC	23 F	62	102	2.75	2.57	+0.18
MS	22 F	64	140	2.73	2.69	+0.04
MR	34 M	66	156	2.78	2.79	−0.01
GB	32 M	72	165	2.68	3.06	−0.38
DG	43 M	64	118	2.72	2.42	+0.30
ER	35 M	67	175	2.35	2.39	−0.04
RM	32 M	70	169	3.93	3.70	+0.23
RMM	20 F	64	116	2.46	2.73	−0.27
ABDB	31 M	75	199	4.30	4.35	−0.05
Mean				2.97	2.97	0.00 S.D. ±0.22 S.E. ±0.07

the open circuit method and 2.97 liters by the plethysmographic method; the standard deviation of the mean of the differences was ± 0.22 liter and the standard error of the mean of the differences was ± 0.07 liter. We conclude that in normal men and women, the open circuit method and the plethysmographic method measure essentially the same gas volume, the functional residual capacity.

4. *Effect of varying volumes of abdominal gas on the thoracic gas volume measured by the plethysmographic method.*

One of the principal objections to the plethysmographic method in the past has been that the gases in the gastro-intestinal tract may be compressed and decompressed along with the gas in the lungs by the voluntary efforts of the subject. By a modification of the technique just presented so that voluntary compression and decompression of the *abdomen* with an open glottis is used instead of respiratory efforts, we have been able to measure the volume of gas present in the abdomen (11); the average value in 60 subjects was 116 ml. This amount of gas could introduce only a very small error in the measurement of the thoracic gas volume by the plethysmographic technique. In order to study the effects of larger volumes of abdominal gas, we have introduced measured amounts of gas into the stomach and colon of normal subjects and studied the effect of this procedure on the value for thoracic gas volume measured by the plethysmographic method. The results (Table II) show that compressible gas volume was unaltered by the addition of large amounts of gas into the stomach and colon. This finding can be explained by one of two mechanisms: 1) The diaphragm was raised and we measured the unchanged total thoracic and abdominal gas volume. 2) The diaphragm was not raised and movement of the diaphragm did not compress and decompress the abdominal gas. On the basis of the data presented in this study we cannot tell which of these two mechanisms is effective when large amounts of gas are introduced into the gastro-intestinal tract.

TABLE II

Effect of gas in stomach and colon on the measurement of resting end-expiratory thoracic gas volume by the plethysmographic method

A. Effects of Adding Gas to Stomach

Subject No. 1 Gas added *ml.*	Thoracic gas volume *L.*	Subject No. 2 Gas added *ml.*	Thoracic gas volume *L.*
Control	4.39	Control	3.51
+200	4.32	+200	3.26
+400	4.28	+400	3.45
+600	4.36	+600	3.57
+800	4.36	+800	3.18
		+1,000	3.27

B. Effects of Adding Gas to Colon

Subject No. 1 Gas added *ml.*	Thoracic gas volume *L.*	Subject No. 2 Gas added *ml.*	Thoracic gas volume *L.*
Control	4.30	Control	3.37
+300	4.45	+300	3.47
+600	4.47	+600	3.28
+900	4.45	+900	3.42
+1,200	4.35	+1,200	3.14
+1,500	4.78		

DISCUSSION

Many methods are available for measuring with reasonable accuracy the functional residual capacity of the lungs and a new method should have definite advantages over existing techniques if it is to become useful for physiological or clinical purposes. These are two major advantages of this new method using a body plethysmograph:

1. The test is the most rapid of present methods and requires no more than a few minutes of the patient's time. No gas samples are needed. The test is easy to perform, requires no unusual cooperation on the part of the subject and is safe. In normal subjects, it gives values which do not differ significantly from the values for FRC obtained by an open circuit washout method. The plethysmographic method can be repeated as frequently as desired and so can be used to follow rapid changes in FRC.

The test does require apparatus not usually found in a pulmonary function laboratory (two sensitive manometers, a body plethysmograph, a shutter and a cathode ray oscilloscope). However, in industrial health departments where it is desirable to make measurements on large numbers of employees, the method, despite the initial expenditure involved, may well be more economical than those involving longer periods of testing and many chemical analyses. Furthermore, the identical apparatus used for this measurement may also be used for the quantitative measurement of airway resistance (9), of pulmonary non-elastic tissue resistance (12), of pulmonary capillary blood flow (13), and of abdominal gas volumes (11).

2. Other methods for measuring FRC measure the volume of pulmonary gas in free communication with the airway. This technique measures the volume of gas within the thorax that is compressed and decompressed with expiration and inspiration. Use of this method simultaneously with a dilution method should permit accurate determination of the volume of non-ventilated gas and lead to a better understanding of this somewhat neglected subject.

CONCLUSIONS

A new plethysmographic method for measuring thoracic gas volume is described. It is rapid, safe, uninfluenced by the usual quantities of abdominal gas, requires no gas samples for chemical analysis and has been found to give reproducible and accurate values which, in normal individuals, are essentially the same as those measured by the open circuit method of Darling, Cournand, and Richards (1). A major advantage of the new technique is that it measures the total volume of compressible gas in the thorax, whether in communication with the airway or not, and therefore should provide the basis for the quantitative measurement of non-ventilated gas.

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A NEW METHOD FOR MEASURING AIRWAY RESISTANCE IN MAN USING A BODY PLETHYSMOGRAPH: VALUES IN NORMAL SUBJECTS AND IN PATIENTS WITH RESPIRATORY DISEASE[1]

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Satisfactory methods, utilizing measurements of transthoracic or transpulmonary pressure and airflow, are now available for determining "non-elastic" pulmonary resistance. However, the non-elastic pulmonary resistance has two components, tissue resistance and airway resistance, and no valid direct method is available for measuring either of these components separately in man. Since airway resistance is the ratio of alveolar pressure during flow to airflow, airway resistance alone could be measured if there were a method for determining alveolar pressure during flow. This report presents a new method for accomplishing this measurement, and gives data for airway resistance obtained in normal subjects and in patients with respiratory disease.

Of previous attempts to measure airway resistance, one of the earliest was the painstaking study by Rohrer (1) who made anatomical measurements on the tracheobronchial tree of a human lung post mortem and calculated the cumulative resistance to airflow of the entire system using Poiseuille's law and turbulence theory. The first important experimental study of pulmonary resistance in the living animal was made by von Neergaard and Wirz who, in 1927 (2), analyzed intrapleural pressure into two major components, "dynamic" (which is essentially resistive) and "static" (which is predominantly elastic in nature). This approach made it possible to obtain values for total pulmonary resistance, though not for airway resistance alone. Bayliss and Robertson (3), reasoning that airway resistance, but not tissue resistance, would vary with the viscosity of the gases breathed, ventilated isolated animal lungs with gases of different density and viscosity; from the changes in pressure and volume during artificial ventilation with different gases, they calculated the fraction of non-elastic pulmonary resistance attributable to airway resistance. Studies based upon the revised principle of utilizing several different gas mixtures of different density and viscosity have been carried out more recently in man by Fry, Ebert, Stead, and Brown (4) and McIlroy, Mead, Selverstone, and Radford (5) who made the point that gas combinations which have equal kinematic viscosities should be selected. The results using this technique have been at variance to date, possibly owing to other factors affecting airway resistance (5).

A number of investigators have measured mouth pressure immediately after interruption of airflow with the belief that in such a static system, mouth pressure equals alveolar pressure. Von Neergaard and Wirz (6), Vuilleumier (7), Otis, Fenn and Rahn (8), and Mead and Whittenberger (9), using such a technique, found that brief interruption of the airstream gave a pressure at the mouth of about the same magnitude as the "dynamic" component of ventilatory pressure; however theoretically the alveolar pressure, after airflow has stopped, is not necessarily the same as before the airflow stopped, because there is adequate time for a major change in alveolar pressure to occur during the transition from flow to no flow (9). There are no measurements which compare alveolar pressure during interruption and during airflow because until now it has not been possible to measure alveolar pressure during airflow.

METHOD

Airway resistance (R) is the ratio of alveolar pressure (P_A) to airflow ($\dot{V}$) at a particular time. Since airflow can be measured readily with a pneumotachograph, the method of measuring airway resistance presented in this

[1] These studies were aided (in part) by a contract between the Office of Naval Research, Department of the Navy, and the University of Pennsylvania, NR 112-323.

paper centers on the determination of alveolar pressure as it exists during airflow. The general principle of the method is as follows: The subject sits and breathes inside an airtight box (body plethysmograph) similar to those which have been used to estimate the volume of gas in the lungs (10) or abdomen (11) by application of Boyle's law relating gas volumes and pressures. If there were no airway resistance, the alveolar pressure would be equal to the ambient pressure in the plethysmograph throughout the respiratory cycle and neither pressure would fluctuate, provided the R. Q. were 1 and there were no change in temperature or saturation of the gas in the plethysmograph-lung system. However, to make gas flow through the airway during expiration, alveolar pressure must exceed box pressure and to make gas flow during inspiration, alveolar pressure must be less than box pressure. Since the total amount of gas in the plethysmograph-lung system is constant, an increase in gas pressure inside the lungs of the subject, as during expiratory effort, must cause a decrease in pressure in the remainder of the gas in the plethysmograph. Therefore, at any instant, the resulting pressure change in the box must be opposite in sign to the pressure change in the lungs. By means of a very sensitive capacitance manometer, the pressure changes in the plethysmograph can be measured continuously during the respiratory cycle.

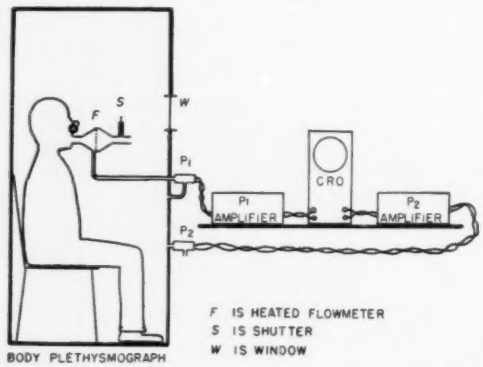

FIG. 1. APPARATUS FOR MEASUREMENT OF AIRWAY RESISTANCE

A. With shutter, S, open, air from plethysmograph is breathed through heated flowmeter, F. Flow is recorded on the Y-axis of the cathode ray oscillograph, CRO, by means of pressure transducer, P_1. Simultaneous change in body volume is recorded on the X-axis of the cathode ray oscillograph by means of pressure transducer, P_2. Alveolar gas is compressed during expiration and expanded during inspiration.

B. With shutter closed, there is no airflow. Mouth pressure (in equilibrium with pressure in the tracheobronchial tree) is recorded on the Y-axis of CRO by means of pressure transducer, P_1. Change in body volume is recorded on the X-axis of CRO by means of P_2. Lung volume is computed by means of Boyle's law for gases.

Airway resistance is computed by combining the results of A (shutter open) and B (shutter closed).

The measured value, change in plethysmographic pressure, can be used to calculate change in alveolar pressure if one also knows the initial alveolar gas volume and the initial alveolar pressure (which is assumed to be equal to atmospheric pressure at end expiration when there is no gas flow). However, this calculation is time-consuming and airway resistance can be determined much more quickly by the following procedures: 1) Changes in airflow are plotted simultaneously against plethysmographic pressure changes (which are proportional to changes in alveolar pressure) on the two axes of a cathode ray oscillograph (CRO). 2) Immediately thereafter, changes in plethysmograph pressure are plotted against mouth pressure on the cathode ray oscillograph under *static* conditions, as the subject makes inspiratory-expiratory efforts against a closed airway; since mouth pressure equals alveolar pressure in a static system, this second step serves to relate changes in plethysmograph pressure to changes in alveolar pressure in each subject. Thus, alveolar pressure is effectively measured during flow since the alveolar pressure for a given plethysmograph pressure is the same whether or not flow is interrupted provided the ratio of lung to plethysmograph gas volume is constant. The difference between this method and previous interrupter methods is that the interruption of flow in the present method is merely the means of calibrating the changes in plethysmograph pressure in terms of alveolar pressure; the values for resistance are always obtained during uninterrupted airflow. The details of the method will now be described:

1. *Measurement of changes in pressure in the body plethysmograph simultaneously with flow:* The plethysmograph is a metal chamber with the proportions of a telephone booth but having an air tight door; its internal volume is approximately 600 liters. Attached to this box is a sensitive Lilly capacitance manometer [2] which responds to a pressure of 0.05 cm. H_2O by one inch horizontal deflection on a cathode ray oscillograph; [3] it measures pressure changes in the box with reference to atmospheric pressure (Figure 1).

The subject sits inside and breathes the air in this closed chamber. The box can be vented to the atmosphere by a valve.[4] At the end of the two-minute warm up time, the subject, who wears a noseclip, places his mouth on a flowmeter-shutter apparatus (which is suspended from the box ceiling by means of curtain rods with spring sockets) and pants to and fro for 5 to 15 sec. through the flowmeter [5] (volume 0.3 liter and sensi-

[2] Lilly capacitance manometer, Technitrol Engineering Co., Philadelphia, Pa.

[3] Cathode Ray Oscillograph, Type 304 A, Allen B. Dumont Co., Clifton, N. J.

[4] Solenoid valve, Automatic Switch Co., Orange, New Jersey.

[5] Flowmeter, Technitrol Engineering Co., Philadelphia, Pa.

tivity 1.34 liters per sec. per in. deflection) which is warmed by an electric current passing through a loop of nichrome wire.

When the subject breathes, airflow is recorded on the Y-axis of the cathode ray oscillograph and plethysmograph pressure (which is proportional to alveolar pressure) is recorded on the X-axis; the slope of the line generated on the cathode ray oscillograph is $\dot{V}/P_P$ where $\dot{V}$ is airflow and P_P is plethysmograph pressure (Figure 2).

Breaths of normal tidal volume produce an artefact caused by the instantaneous warming and wetting of inspired air and the cooling and condensation of expired air; because the rate of the latter is less than of the former, this produces a net effect in the direction of increased pressure in the box with each breath. Shallow breathing through the heated flowmeter reduces the size of the artefact. As long as the front between plethysmograph air and pulmonary air remains inside the metallic heated flowmeter, this artefact is eliminated. This artefact is also eliminated when the subject rebreathes in a rubber bag containing hot water, which serves to keep the respired air at constant conditions of temperature and saturation throughout the respiratory cycle.

Although most previous investigators using other methods have measured resistance to breathing at a normal breathing frequency and tidal volume, rapid, shallow breathing such as panting has certain theoretical and practical advantages: It minimizes the temperature, saturation and R. Q. effects to the extent that they can be neglected. It improves the signal to drift ratio because it allows completion of each respiratory cycle in a fraction of a second. Gradual thermal changes and very small leaks in the box become insignificant compared to the volume changes attributable to compression and decompression of alveolar gas.

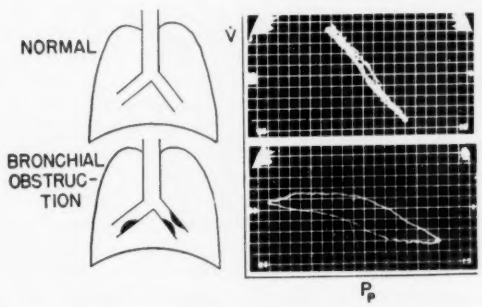

FIG. 2. PHOTOGRAPH OF CATHODE RAY SCREEN (SHUTTER OPEN)

Airflow on the Y-axis has calibration of 1.3 L. per sec. per inch deflection. Change in body volume on the X-axis has calibration of 14 cc. per inch deflection (normal subject), and 27 cc. per inch, case No. 20, bronchial obstruction. Zero flow is in center of screen. Although a simple 'S' shaped resistance line was characteristic of normal subjects, patients who had predominantly expiratory obstruction generally showed the looping of resistance, which was increased in association with expiratory flow, or volume, as in this case, or both.

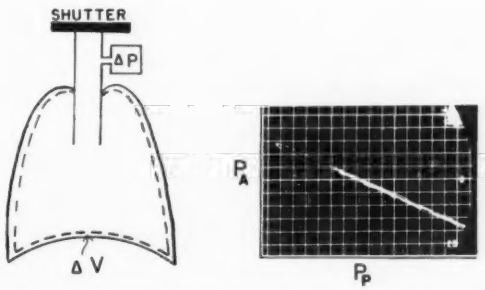

FIG. 3. PHOTOGRAPH OF CATHODE RAY SCREEN (SHUTTER CLOSED)

Mouth pressure on Y-axis has calibration of 7.1 cm. H_2O per inch deflection. Change in body volume on the X-axis has calibration of 14 cc. per inch deflection. Atmospheric pressure in center of screen. Grid lines in Figure 2 and Figure 3 are 0.2 inch.

Certain objections may be raised to the use of panting: a) The volume of gas in the lungs during panting is often different from the volume during quiet breathing because the subject chooses a lung volume which offers the least sensation of obstruction to breathing, b) the subject may tend to open maximally his upper airway (mouth and glottis), c) the subject breathes so shallowly that gas distribution is not limited by stiff regions of the lung, but the gas follows only the pathways of least resistance and d) panting at very low tidal volumes leads to a rise in P_{CO_2} and a decrease in P_{O_2} in the airways during the period of the test. On the other hand it has been shown that the over-all resistance to breathing can be measured by a pump at 6 cycles per second (12), that the frequency response of the chest wall is satisfactory in this frequency range (13) and that respiratory resistance measured in cats is not different when measured at high and low frequencies (14). However, further studies are needed to determine the exact magnitude of changes in airway resistance produced by the panting procedure *per se*. The present measurements of airway resistance were made at panting frequencies which were approximately 2 cycles per second. This frequency is well within the limit of both capacitance manometers since their frequency responses were flat to at least 35 cycles per second.

2. *Method of relating plethysmographic pressure changes to alveolar pressure changes:* To relate changes in plethysmograph pressure to changes in alveolar pressure, the following procedure is performed: The operator activates a rotary solenoid (9) which occludes the distal end of the flowmeter. The subject continues to pant and records are made continuously of mouth pressure (Y axis) and plethysmograph pressure (X axis) (Figure 3). (The pressure gauge[6] formerly used to record differential

[6] Venous pressure head, Technitrol Engineering Co., Philadelphia, Pa.

pressure across the flowmeter now measures mouth pressure with respect to box pressure. Ideally, it should now measure mouth pressure with respect to atmospheric. The 1 per cent error was insufficient to warrant the technical difficulty of changing over the back side of the gauge. However it is necessary to reduce the sensitivity of this gauge by a factor of ten at the same time that the rotary solenoid is activated; its sensitivity is now 7 cm. H_2O per inch deflection.) Since there is no airflow during the period of occlusion, mouth pressure equals alveolar pressure and the slope of the line generated on the cathode ray oscillograph is now P_A/P_P where P_A is alveolar pressure and P_P is plethysmograph pressure. Since the same manometer is used to record P_P both with the shutter open and closed, P_P of both slopes is the same.

The lines produced on the oscilloscope are traced [7] or photographed (f 5.6, bulb exposure, Plus-X film, with plus 2 diopter supplementary lens) or both. The total time in the box is approximately 5 minutes. The flowmeter is then calibrated with a recording spirometer to measure airflow and the mouth pressure gauge is calibrated with a water manometer.

3. *Calculation of airway resistance:* To determine the airway resistance, it is necessary to know the ratio of alveolar pressure to airflow at the mouth, P_A/V. The experiments yielded two slopes. First, with the shutter open, the slope $\dot{V}/P_P$ was measured on the S shaped line from the point of 1 liter per second of inspiratory flow to the point of 1 liter per second of expiratory flow.[8] Second, the slope P_A/P_P is generated with the shutter closed. The total airway resistance is calculated by taking the ratio of slopes:

$$R = \frac{P_A/P_P}{\dot{V}/P_P}$$

where P_A is alveolar pressure, $\dot{V}$ is airflow, P_P is plethysmograph pressure (the same with shutter open and closed), and R is resistance.

A correction is made on the total airway resistance for the volume of the dead space by assuming that the average resistance to airflow is halfway down the tracheobronchial tree: The slope P_A/P_P is multiplied by the ratio of total gas volume occluded by the shutter to gas volume proximal to the point of mean resistance to gas flow. The total volume is measured immediately at the end of the test by the plethysmographic method (15). The volume proximal to the resistance is the total volume minus the volume of the flowmeter (0.3 liter) and half the volume of the tracheobronchial tree (0.1 liter). Resistance of the flowmeter-shutter breathing apparatus is measured (0.5 to 0.7 cm. H_2O per liter per sec.) and subtracted from the total airway resistance to obtain airway resistance of the subject. The values for airway resistance are expressed in cm. H_2O per liter per sec.

4. *Experimental accuracy:* The method of tracing and reading the image from the cathode ray oscillograph permits a 5 per cent error of slope. Since two different slopes are used in the calculation of resistance, the sum of the errors may be 10 per cent. There is a slight reading error in calibration of the flowmeter and mouth pressure gauge; however errors in calibration of the box pressure gauge are automatically cancelled in the calculation of resistance. Since these reading errors are of a random nature, the over-all accuracy is improved by repeating the measurement of airway resistance several times and averaging the results.

The advantage of tracing is that it permits calculation of the airway resistance without delay; however the slopes can be read more accurately from a photograph than from a pencil tracing.

5. *Validity:* The validity of the plethysmograph method for airway resistance was tested by determining the total resistance before and after various degrees of resistance were interposed between the flowmeter and the subject's mouth. The added resistances were 2.7, 5.9, and 10.9 cm. H_2O per liter per sec.; these caused an increase in airway resistance as measured by the plethysmograph method of 2.2, 5.9, and 9.3 cm. H_2O per liter per second, respectively. The discrepancies were within the combined reading errors of the methods for measuring the values of the added resistances and the airway resistance.

6. *Other possible sources of error:* Since the total gas volume, which is used to correct for the volume of the dead space in calculating resistance is determined by a plethysmograph method (15), a gross check of this method was done as follows: The total gas volume was determined before and after the subject inspired measured volumes from a recording spirometer. The inspired volumes were 1.1 liters, 2.4 liters, and 3.7 liters and the compressible gas was found to increase by 1.0 liters, 2.2 liters, and 3.5 liters, respectively. The presence of abdominal gas might conceivably produce an error in the calculated volume of compressible gas in the lungs. If the abdominal gas were compressed during expiration and expanded during inspiration, the total apparent compressible volume would be greater than the lung volume, whereas if the abdominal gas were expanded during expiration and compressed during inspiration, the calculated volume would be less than the lung volume. However, if the calculated gas volume during voluntary efforts against the closed shutter were equal to the calculated volume during voluntary panting through the flowmeter, there would be no error in the calculation of airway resistance, because the plethysmograph pressure terms which would be affected cancel during the calculation. Errors in box calibration are eliminated for the same reason. Abdominal pressure changes measured by a gastric balloon and box pressure were recorded on two normal subjects and four patients during panting through

[7] "Oscillotracer," R. A. Waters, Inc., Waltham, Mass.

[8] Some subjects showed increased end expiratory resistance, forming a loop in the appropriate portion of the pressure-flow curve. This loop was probably caused by partial or complete collapse of some of the air passages as the subject expired toward residual volume. In these subjects the mean slope was measured after drawing a line along the imaginary center, or long axis of the loop

the flowmeter and against the closed shutter and the relationship between abdominal and box pressure showed no significant difference with the shutter open or closed. The calculated volume, shutter-open, would therefore be the same as with the shutter closed. The conclusion is that abdominal gas cancels out in the calculation of airway resistance. More detailed accounts of the accuracy of the plethysmographic method of determining thoracic gas volume and abdominal gas volumes will be the subject of separate reports (15, 16).

The R. Q. might conceivably produce an error in the determination of airway resistance by the plethysmographic method since there is a gradual absorption of gas when the mean R. Q. is less than 1.0. For example, if oxygen consumption is 300 cc. per minute and the CO_2 output is 240 cc. per minute the mean R. Q. is 0.8 and the net gas absorption is 60 cc. per minute ("respiratory exchange difference") or 1 cc. per sec. During inspiration, the respiratory exchange difference is slightly greater, and during expiration, it is slightly less than the mean. Upon rebreathing the dead space gas, the respiratory fluctuations disappear, but the trend of the respiratory exchange differences changes, as in breath holding, causing a slight increase in the net rate of gas absorption. Neither the mean trend nor the respiratory fluctuations are large enough to interfere with the measurement of airway resistance or lung volume because, during panting, pressure changes are sufficiently rapid and the volume changes due to compression of lung air are large enough that the R. Q. effect is small by comparison, even in subjects with normal airway resistance.

7. *Other tests:* Those performed on the patients included vital capacity, maximal expiratory flow rate over the range 200 to 1,200 cc., maximal breathing capacity using a Tissot spirometer and lung compliance using an esophageal balloon, capacitance manometer, spirometer with potentiometer, and cathode ray oscillograph.

RESULTS AND DISCUSSION

1. *Normal subjects*

Twenty-one subjects who had no signs, symptoms, or history of respiratory disease were studied; ten of these were tested on two different days, and eleven were tested on only one day. The mean airway resistance of the 21 normal subjects was 1.5 cm. H_2O per liter per sec., at a flow of one liter per second, panting. The standard deviation was $\pm$ 0.49 and the range 0.6 to 2.4 (Table I). In those subjects tested on two different days, the standard deviation of the mean of the differences between determinations was $\pm$ 0.37. Because this small series contains men and women in all age groups, breathing at different lung volumes, these data are presented only to give an approximate range of normal values; larger groups in all decades are now being studied in order to define normal values more precisely.

In this series, the sensitivity of the manometers was adjusted so that the most accurate readings

TABLE I

Airway resistance in normal subjects (panting) *

Subject	Age (*yrs.*)	Sex	Height (*ft., in.*)	Weight (*pounds*)	Plethysmographic thoracic gas volume (*liters, BTPS*)		Airway resistance (*cm. H_2O/L./sec.*)	
					No. 1	No. 2	No. 1	No. 2
1. F. K.	33	M	5′10″	165	3.9	3.9	1.6	1.3
2. J. M.	32	M	5′5″	133	2.4	2.8	1.6	1.3
3. L. C.	28	M	5′9½″	180	5.3	1.8	1.4	1.7
4. E. H.	30	F	5′4¾″	137	2.0	2.3	2.1	1.7
5. L. D.	28	M	5′7″	160	3.0	2.7	1.6	1.8
6. P. C.	23	F	5′3″	101	2.4	2.8	1.8	1.1
7. M. E.	22	F	5′10″	145	3.5	4.0	1.4	1.3
8. A. V.	40	M	6′½″	190	3.4	5.2	1.1	0.7
9. C. C.	29	M	5′9″	145	3.6	3.5	2.0	1.7
10. A. D.	30	M	6′3″	200	2.9	3.7	1.3	1.8
11. B. L.	29	M	5′11″	175	5.2	—	1.4	—
12. J. T.	29	F	5′5″	110	1.9	—	1.9	—
13. D. T.	28	F	4′10″	93	2.4	—	2.4	—
14. E. B.	37	M	5′10½″	160	4.5	—	0.6	—
15. M. J.	51	M	5′11″	185	4.2	—	0.9	—
16. S. D.	57	M	5′3″	150	3.0	—	2.3	—
17. R. N.	37	M	5′11″	150	3.7	—	1.7	—
18. M. M.	33	M	6′3″	185	3.9	—	1.0	—
19. R. C.	51	M	5′10½″	168	5.4	—	0.9	—
20. C. M.	35	M	6′1″	150	4.8	—	1.9	—
21. R. D.	27	F	5′8½″	117	3.5	—	1.3	—

* Subjects 1–21, mean airway resistance = 1.50 cm. H_2O per L. per sec., S.D. $\pm$0.49. Subjects 1–10, mean of the differences between No. 1 and No. 2 measurements of airway resistance = 0.15 cm. H_2O per L. per sec., S.D. $\pm$0.37.

of the S-shaped curve on the cathode ray oscillograph were made at flow rates of 1 liter per sec. Mean values of airway resistance measured at flow rates of 0.75 liter per sec. are approximately 0.1 cm. H_2O per liter per sec. less and those measured at 0.5 liter per sec. are approximately 0.2 cm. H_2O per liter per sec. less than values obtained at flow rates of 1.0 liter per sec. The values obtained represent those for a specific pattern of mouth breathing; the resistance to airflow through the nasal passages may be appreciable as indicated by measurements of pressure differential between nasopharynx and one nostril, which we have found to range from 1 to 4 cm. H_2O per liter per sec. at

TABLE II

Airway resistance in thirty patients

Subject	Age (*yrs.*) and sex	Disease	Duration of disease (*yrs.*)	Chief symptom*	Vital capacity (*L.*)	Max. expir. flow rate (*L./min.*)	Max. breathing capacity (*L./min.*)	Lung compliance (*L./cm. H_2O*)	Plethysmographic thoracic gas volume (*L.*)	Airway resist. (*cm. H_2O/L./sec.*)	Measured at flow rate of (*L./sec.*)
1. W. M.	42 M	? Silicosis	2	DOE, slight	4.6	530	168	0.22	3.9	1.9	1.0
2. E. M.	48 F	Scleroderma	3	Cough	2.1	170	114		3.0	1.6	1.0
3. R. D.	44 M	Scleroderma† (biopsy)	3	Dyspnea, occasional	1.5		145	0.04	1.6	0.6	0.9
4. M. C.	44 F	Chr. bronchitis‡		DOE	2.4			0.15	3.5	5.5	0.8
5. J. L.	65 F	Asthma‡	31	DOE, 1 flt.	2.5	72		0.08	4.1	9.8, 7.0§	0.7
6. J. C.	55 M	Asthma and stenosis of the glottis‡	25	Parox. dyspnea	4.5	124	43		3.5	7.2	1.0
7. N. P.	56 M	Chr. bronchitis‡	18	DOE and cough	3.4	93	44		7.3	3.4	1.0
8. W. H.	54 M	Asthma‡	21	DOE	3.3	29		0.26	6.7	5.2	0.9
9. I. B.	67 M	Asthma	12	Dyspnea at rest	1.8	22	22	0.06	6.5	6.8 1.9‖	0.58
10. J. J.	56 M	Asthma	37	DOE, 1 flt.	2.4	17			3.7	6.8	0.68
11. A. A.	64 F	Asthma	15	DOE, occasional	2.0	115			2.8	3.1	0.5
12. C. C.	54 M	Pulm. fibrosis†‡	1	"Chest cold"	1.2	260	50	0.011	2.6	2.8	0.7
13. S. T.	21 M	Asthma		Asympt.	5.5	270		0.21	2.9	3.2	0.8
14. C. K.	63 F	Asthma	12	Dyspnea at rest	1.5	58		0.05	2.6 2.3	6.8§ 6.8	0.7
15. B. J.	38 M	Asthma	30	Dyspnea at rest	1.9	13		0.06	5.6 5.5	7.5§ 6.9	0.5
16. R. H.	51 M	Asthma		Dyspnea, slight	2.1	44		0.13	6.1	5.9	0.8
17. L. B.	53 M	Asthma	47	Dyspnea, occasional	2.6	35		0.27	5.1	4.5	0.9
18. E. N.	42 F	Asthma		Asympt.	2.2	77	43	0.20	3.5	3.9	0.7
19. G. D.	53 M	Emphysema	3	DOE, slight	2.6	66		0.11	5.6	7.7	0.5
20. W. L.	51 M	Silicosis	11	DOE, 1 flt.	2.1	19	26	0.14	5.1	10.8	0.5
21. S. S.	63 F	Asthma		Asympt.	2.2	49		0.08	3.9	8.3	0.7
22. B. J.	28 M	Asthma	10	Dyspnea	2.3	19			6.0	4.7	0.9
23. M. H.	9 F	Pulm. fibrosis	1	Dyspnea, ½ flt. Resp. rate 72/min.	0.2				1.0	5.2	0.6
24. J. H.	61 M	Pneumoconiosis	9	DOE, 1 block	2.4	26	60		4.6	6.4	0.5
25. M. C.	62 M	Pneumoconiosis	2	DOE, 1 flt.	3.1	99	46		4.8	2.3	0.5
26. H. P.	44 M	Asthma	16	Asympt.	2.6	132			3.2	2.2	0.9
27. E. C.	47 F	Hay fever	30	Asympt.	2.8	140			3.2	2.2	0.5
28. G. L.	58 M	"Alveolar cell" Ca†	1	DOE, severe	0.9		72	0.013	2.1	2.2	0.7
29. L. F.	55 M	Asthma ?	17	Asympt.	1.9	57?		0.12	4.1	1.7	0.7
30. E. W.	26 F	Normal preg.	0.7	Asympt.					3.7	2.6	1.0

* DOE = Dyspnea on exertion; flt. = flight.

† Biopsy of lung showed: No. 3, interstitial fibrosis; No. 12, pulmonary fibrosis; autopsy showed: No. 28, no tracheobronchial obstruction.

‡ Bronchoscopy showed: No. 4, chronic tracheobronchitis; No. 5, bronchiectasis and purulent tracheobronchitis; No. 6, stenosis of glottis (laryngoscopy); No. 7, bilateral tracheobronchitis; No. 8, allergic tracheobronchitis; No. 12, no abnormalities.

§ Patients 5, 14 and 15, airway resistance measurement was repeated on a subsequent day.

‖ After subcutaneous injection of epinephrine.

flow rates of 0.25 liter per sec. in normal subjects with no subjective complaints of nasal obstruction. Therefore the resistance of the total airway in subjects breathing through the nose would be greater than the values obtained in this study. The resistance predicted by Rohrer from anatomical measurements of the tracheobronchial tree in a cadaver was 1.6 cm. H_2O per liter per sec. It is not possible to make a comparison of these data with data obtained by other investigators using different methods for several reasons: a) Some of the methods purporting to measure airway resistance alone actually include other factors as well and are not specific tests of airway resistance, b) the specific breathing pattern used in this test differs from that used by other investigators. However, a comparison with the esophageal pressure method for pulmonary resistance, reported in detail in reference 17, yielded mean normal pulmonary tissue resistance of approximately 0.2 cm. H_2O per liter per second at 0.5 liter per second.

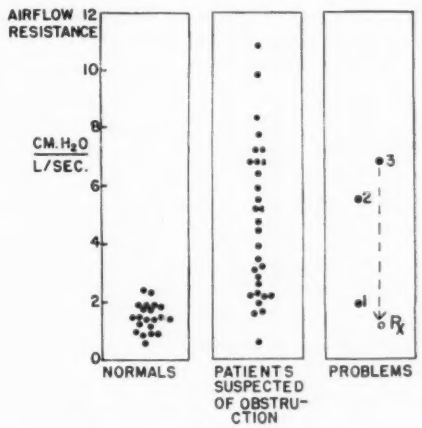

FIG. 4. SUMMARY OF AIRWAY RESISTANCE VALUES IN NORMAL SUBJECTS, PATIENTS WITH SUSPECTED OBSTRUCTION AND IN THREE PROBLEM CASES

The three problem cases are as follows:

1. W. M. Case No. 1 (Table II), was referred for evaluation of possible pulmonary disability due to suspected pneumoconiosis: Objective pulmonary function tests were of value in ruling out impairment of function.

2. M. C. Case No. 4 (Table II), was tested to distinguish between dyspnea accompanying anxiety versus organic disease. This method revealed evidence of organic tracheobronchial obstruction.

3. I. B. Case No. 9 (Table II), was evaluated for effect of bronchodilator drugs on respiratory obstruction. It was found that his obstruction was not of the 'fixed' type, but reversible by administration of epinephrine subcutaneously.

2. Patients with respiratory disease

No attempt will be made here to present a complete analysis of the mechanical factors in breathing in patients with respiratory disease. However this test for airway resistance has been applied to a sufficient number of patients to evaluate its practicality, limitations and usefulness in diagnosis and treatment.

The method cannot of course be used in patients who are too sick or too weak to sit in the plethysmograph. Patients with bilateral paralysis of the lower extremities would have to be lifted in and out of the box. No patients refused to enter the box but one "normal" subject refused to enter the box because of claustrophobia. Several patients were unable to breathe at flow rates as great as 1.0 liter per sec.; in these the resistance was calculated at flow rates of 0.5 to 1.0 liter per sec. Many of the patients with high resistance showed loops similar to that in Figure 2, bottom, compatible with expiratory obstruction. The 30 patients, including one child aged nine years, studied in this series were all able to follow directions and complete the test satisfactorily. Data obtained upon these 30 patients are presented in Table II and Figure 4. Since there is no other method available for direct measurement of airway resistance, the reliability of this method cannot be checked experimentally except for evidence of false positive or false negative indication of tracheobronchial obstruction. For example, since patients 1 to 3 had a maximal breathing capacity in the normal range, it is unlikely that they had any significant increase in airway resistance; their values (0.6 to 1.9 cm. H_2O per liter per sec.) fell in the normal range. Patients 4 to 8 had known partial obstruction in their large airways by bronchoscopic examination and all had increased airway resistance by the present test.

The values for airway resistance in patients 9 to 24 were increased and in all of these there were clinical reasons for expecting either increased airway or tissue resistance. Patients 25 to 28 were borderline cases with high normal values for airway resistance and clinical findings suggestive of

partial obstruction. Patient 29 had airway resistance within one standard deviation of the normal mean, but his maximal expiratory flow rate was less than normal. This means that the low value for maximal expiratory flow rate was caused by factors other than increased airway resistance; some of these might be partial collapse of the intrathoracic portion of the tracheobronchial tree during forced expiration (which would not occur with the present test), by increased resistive factors in the chest wall or lung tissues, or by failure to exert maximal force. The finding of slightly increased airway resistance in patient 30 is not readily explained although it is possible that this slightly increased airway resistance might be the result of distortion of the airways as a result of the pregnancy; further studies must be done on a large group before any conclusions can be drawn.

No attempt will be made to correlate airway resistance measured by this test with maximal breathing capacity, timed vital capacity, or maximal expiratory flow rates, since these last named tests are "over-all" tests of mechanical factors during forced breathing and include many factors in addition to the specific airway resistance being measured here.

It appears that this method of measuring airway resistance alone has the following uses: a) Objective measurement of airway resistance in patients; it has the advantage of measuring one specific component of the mechanical factors in breathing, without the requirement of maximal respiratory effort on the part of the patient; b) Quantitative and objective evaluation of therapeutic procedures designed to relieve airway obstruction; c) Separate measurement of airway resistance so that tissue resistance can be determined as the difference between total resistance and airway resistance; d) Study of multiple physiological, pharmacological and environmental and pathological factors that may affect airway resistance.

SUMMARY

A new method is reported for the objective and specific measurement of airway resistance in human subjects. It requires the measurement of airflow and of alveolar pressure *during airflow;* the latter is measured by determining by means of a body plethysmograph the volume of compression and decompression of alveolar gas during expiration and inspiration.

Normal subjects tested by this method had a mean airway resistance of 1.5 cm. H_2O per liter per sec. (range 0.6 to 2.4) at a flow rate of 1 liter per second, panting. A preliminary study has been made of the airway resistance in 30 patients; the range of resistances was 0.6 to 10.8 cm. H_2O per liter per sec.

This method is thought to be applicable to measurement of airway resistance in patients, evaluation of therapeutic procedures designed to relieve airway obstruction, separation of airway resistance from tissue resistance, and study of multiple factors that may affect airway resistance.

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MEASUREMENT OF THE VOLUME OF GAS IN THE GASTRO-INTESTINAL TRACT. VALUES IN NORMAL SUBJECTS AND AMBULATORY PATIENTS[1]

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During the course of lung volume studies it became necessary to determine the effect of gases in the abdomen on the thoracic gas volume as measured by a plethysmographic method. Blair, Dern, and Bates (1), using a plethysmographic method for the determination of abdominal gas volume, reported that the average volume of gas present in the gastro-intestinal tract was approximately one liter. No other studies have been reported either to confirm or to contradict these results and the purpose of this paper is to describe an improved plethysmographic method for the determination of abdominal gas volume and to report the results obtained in normal subjects and in ambulatory patients with pulmonary disease. The average volume of abdominal gas is of interest as it relates to gastroenterology, decompression and measurement of the specific gravity of the body.

The principle upon which the plethysmographic method is based is that the volume of a gas in a closed space in the body varies in inverse proportion to its pressure provided that the temperature remains constant (Boyle's law). If the pressure change in a volume of gas is small in relation to the initial pressure then the initial volume V can be calculated from the equation:

$$V = \frac{\Delta VP}{\Delta P}$$

where P is the initial pressure and ΔP and ΔV are the induced changes of pressure and volume, respectively (2). This equation has been applied to the measurement of abdominal gas volume. The initial pressure is usually a few centimeters of water above atmospheric pressure but the difference is so small in relation to atmospheric pressure that it may be neglected. The change of pressure in the abdomen resulting from voluntary straining is measured by means of an intragastric balloon and changes of pressure in the stomach are considered to be representative of changes in pressure throughout the abdominal cavity. This is an assumption subject to proof but if it is true, volumes of gas introduced into the colon or small bowel should be measured as accurately by recording of intragastric pressure as volumes of gas introduced directly into the stomach. The change in volume of the abdominal gas is measured by use of the body plethysmograph.

The first objective in these studies was to determine the degree of reproducibility and accuracy of measurement of volumes of gas introduced into the stomach. Secondly, it was necessary to establish whether the method could measure accurately volumes of gas introduced into the colon. If this were possible then the theory that the abdominal cavity acts as a unit, with pressures generated by contraction of the abdominal muscles and diaphragm equally transmitted to all parts, would be substantiated. Thirdly, the volume of gas normally present in the gastro-intestinal tract was measured.

METHOD

The apparatus used is shown diagrammatically in Figure 1. The body plethysmograph, B, which has been described previously (2, 3) is a chamber of about 600 liters' capacity and having an airtight door. The pressure differential between the chamber and the atmosphere is measured by a sensitive differential capacitance manometer C, the output from which is made to record on the X axis of a cathode ray oscilloscope O. The sensitivity

[1] These studies were aided (in part) by a contract between the Office of Naval Research, Department of the Navy, and the University of Pennsylvania, NR112-323.

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of the manometer and the amplification of the output are such that a pressure change of 0.025 cm. H_2O in the plethysmograph produces a deflection of 1 inch on the X axis of the oscilloscope. The pressure changes in the closed plethysmograph are proportional to volume changes of the air in the plethysmograph and the manometer is calibrated directly in terms of volume change by recording the deflection produced on the oscilloscope when a known volume of air is introduced into, and withdrawn from, the plethysmograph by a diaphragm pump of 30-ml. stroke volume. The calibration is carried out when the subject is in the plethysmograph.

The pressure in the stomach is measured by use of an intragastric balloon of the type used for intra-esophageal pressure measurements. This is a thin latex balloon, approximately 10 cm. long and 1 cm. wide, attached to a length of 1 mm. bore polyethylene tube. The tube is connected to a capacitance manometer, P, outside the plethysmograph and the output from this manometer is recorded on the Y axis of the oscilloscope. The sensitivity of this manometer is such that 5 cm. H_2O produces a deflection of 1 inch on the oscilloscope. It is calibrated against a water manometer.

The procedure for measurement of the abdominal gas volume is as follows. The balloon is passed through the nose and swallowed until the tip is about 60 cm. from the nares. The location of the balloon in the stomach can be verified by observing the pressure in the balloon when the subject sniffs. This maneuver momentarily increases the pressure in the abdomen and lowers the pressure in the thorax.

The subject is seated in the plethysmograph and approximately 2 ml. of air are introduced into the intragastric balloon. About 2 minutes must be allowed between closing the plethysmograph and making the measurements of abdominal gas so that the temperature and humidity in the plethysmograph may become stabilized. The plethysmograph is opened periodically to the atmosphere through a solenoid operated vent so that the pressure remains approximately atmospheric.

The subject is instructed to stop breathing at the end of a normal expiration but to keep his airways open. He is then instructed to tense the abdominal muscles by "pushing out against the belt," or against the hands placed firmly on the abdomen, in rapid repeated movements. This maneuver is directed towards increasing the pressure in the abdomen without changing the pressure of the air in the thorax. The tracing obtained on the oscilloscope screen is a sloping straight line repeated with each increase of abdominal pressure (Figures 2a and b). This tracing may be recorded either by use of an Oscillotracer [4] or by photographing it, using a still camera with a bulb exposure.

CALCULATION

If the equation shown above is applied to the measurement of abdominal gas volume, then the initial volume

[4] R. A. Waters, Inc., Waltham, Mass.

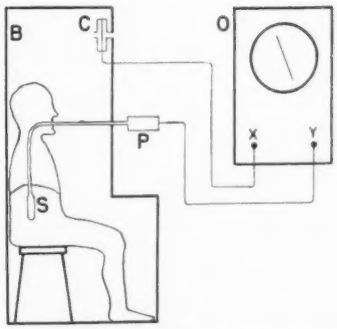


FIG. 1. DIAGRAM OF THE APPARATUS

B, body plethysmograph; C, pressure gauge; P, pressure gauge; S, intragastric balloon; O, cathode ray oscilloscope.

of abdominal gas, V, is given by

$$V = \frac{\Delta V\ 970}{\Delta P}\ \text{ml.}$$

970 (cm. H_2O) is atmospheric pressure minus the pressure of water vapor at 37° C since water vapor tension in the gastro-intestinal tract is presumed constant. The Y axis of the oscilloscope is calibrated in terms of pressure change in the stomach and the X axis is calibrated directly in terms of volume change in the plethysmograph. Thus the slope of the line traced on the oscilloscope is proportional to $\Delta P/\Delta V$ so that, adding the calibration factors, the above equation may be rewritten

$$V = \frac{970\ Cv}{Cp\ \lambda}\ \text{ml.}$$

where Cv is the calibration of volume change in the plethysmograph expressed as ml. per 1-inch deflection, Cp is the calibration of pressure change expressed as cm. H_2O per 1-inch deflection and λ is the slope of the oscilloscope tracing.

Unsatisfactory tracings may be produced in two ways, either by closure of the glottis or by production of airflow. In either case the use of a flowmeter aids in the detection of the artefact. If the subject closes the glottis, then the thoracic gas is also compressed during the maneuver for compression of abdominal gas. When this happens the slope of the tracing is much greater (see Figure 2c) since a very much larger volume of gas is being compressed, and also the cardiac fluctuations usually seen on the flowmeter disappear when the glottis is closed. Unsatisfactory tracings may be obtained if the subject inspires or expires during the maneuver. If the subject inspires as the abdomen is compressed the alveolar pressure is lowered and the inspired air is expanded as it is warmed and humidified. Both of these effects will tend to increase the pressure in the plethysmograph (whereas compression of abdominal gas decreases the pressure in the plethysmograph) and a falsely low value for abdominal gas volume will be obtained. A falsely high value

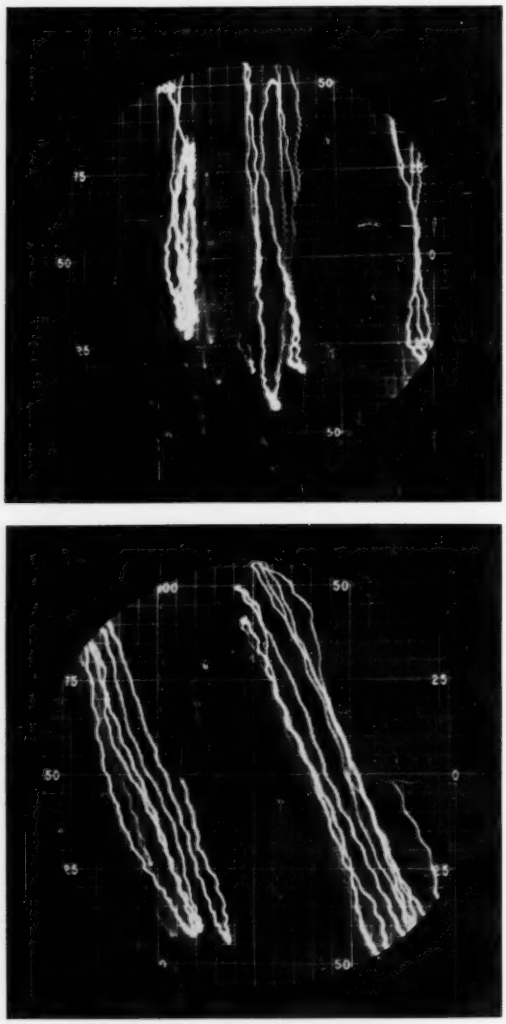


FIG. 2.

will be obtained if the subject expires. In either case the flowmeter tracing will show evidence of airflow.

Even after practice, some subjects are unable to produce satisfactory tracings and the results in 7 out of 67 subjects in this series had to be discarded for this reason. A mechanically driven belt, designed alternately to compress and release the abdomen, was tried in efforts to make the method less dependent on the subject's cooperation but the device proved unsatisfactory due to some slight compression of lung gas.

Measurement of gas volumes added to the stomach. Two polyethelene tubes, one with balloon attached, the other open, were tied together and passed into the stomach. The volume of gas present in the abdomen was determined by the plethysmograph method. This figure was used as a control reading. Increments of 200 ml. were then added through the second tube by means of a syringe. The volume of abdominal gas was measured after each increment. Radiographs of the abdomen were taken before the addition of air and after the introduction of 500 ml. of air into the stomach.

Measurement of gas volumes added to the colon. The stomach balloon was placed as previously described. A rectal catheter was used to inject gas into the colon. This catheter is of the type used to administer barium enemas. It has an inflatable balloon of 30-ml. volume near the tip to prevent its escape from the rectum. The balloon also helped to prevent escape of gas from the colon. Control measurements were made and then air was added to the colon in increments of 300 ml. The volume of abdominal gas was measured after each increment by the same technique of measuring changes in intragastric pressure and plethysmographic volume during contraction of the abdominal muscles. While introducing gas into the colon in subjects R. M. and A. B. D. B. the pressure in the colon was recorded between cramps with an aneroid manometer before and after the introduction of each volume of gas and the circumference of the abdomen was measured by a tape placed at the level of the umbilicus. Radiographs of the abdomen were taken before the addition of air and after the introduction of 600 and 1,200 ml. of air into the colon.

RESULTS

1) *Accuracy of measuring volumes of gas in the stomach*

A total of 38 gas volumes were introduced into the stomach in the two subjects. Figure 3 is a

FIG. 2. PHOTOGRAPHS OF THE OSCILLOSCOPE TRACE WITH INTRAGASTRIC PRESSURE RECORDED ON THE Y AXIS AND PLETHYSMOGRAPHIC PRESSURE ON THE X AXIS

A: Control showing little or no gas; B: After the addition of 1,200 ml. of air to the colon; C: Tracing of mouth pressure (Y axis) and plethysmographic pressure (X axis) with subject panting against a closed shutter. Shown to demonstrate the slope of the tracing when lung gas is compressed.

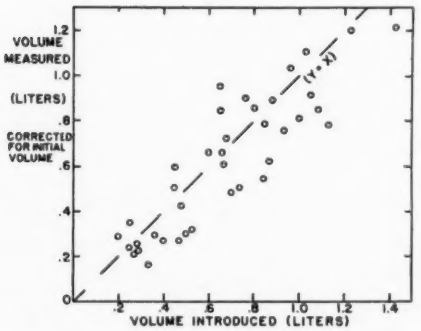


FIG. 3. GRAPHS SHOWING THE RELATIONSHIP BETWEEN THE VOLUME OF GAS INTRODUCED INTO THE STOMACH AND THE INCREASE IN ABDOMINAL GAS VOLUME AS MEASURED BY THE PLETHYSMOGRAPHIC METHOD

Thirty-eight measurements in subjects R. M. and A. B. D. B.

scatter diagram of these data. The volume introduced is on the abscissa, and measured volume minus the initial control volume is plotted on the ordinate. It can be seen that the grouped data are sufficiently linear to warrant application of a linear regression equation to the data of individual experiments (Figures 4 and 5).

Figure 4 shows this plot from an experiment in which stomach gas was introduced into the subject R. M. The measured initial volume is 84 ml., the Y intercept is 6 ml. The slope of the line of regression is 1.0. The standard error of estimate is 91 ml.

Figure 5 shows a similar plot from an experiment in which stomach gas was introduced into subject A. B. D. B. The measured initial volume is 48 ml., the Y intercept is 32 ml. The slope of the line of regression is 1.43. The standard error of estimate is 67 ml.

The radiograph of the stomach after the injection of 500 ml. of air (Figure 8) shows that this volume is many times that usually present in the stomach and that it produces considerable gastric distension.

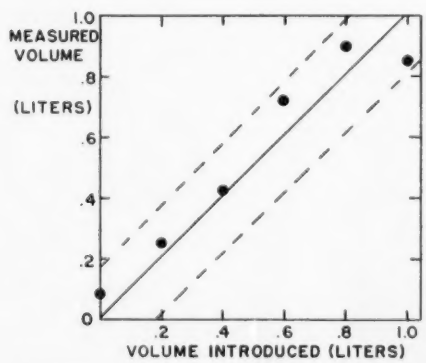


FIG. 4. GRAPH SHOWING THE RELATIONSHIP BETWEEN THE VOLUME OF GAS INTRODUCED INTO THE STOMACH AND THE INCREASE IN ABDOMINAL GAS VOLUME AS MEASURED BY THE PLETHYSMOGRAPHIC METHOD

Results of 1 experiment on subject R. M. Dashed lines represent two times the standard error of estimation.

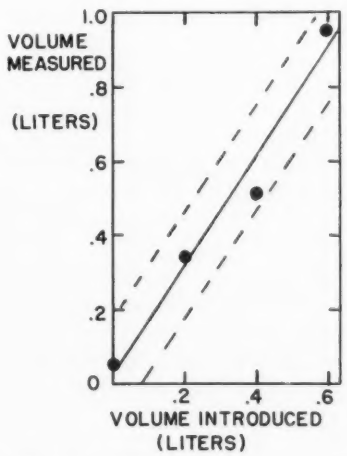


FIG. 5. GRAPH SHOWING THE RELATIONSHIP BETWEEN THE VOLUME OF GAS INTRODUCED INTO THE STOMACH AND THE INCREASE IN ABDOMINAL GAS VOLUME AS MEASURED BY THE PLETHYSMOGRAPHIC METHOD

Results of 1 experiment on subject A. B. D. B.

2) *Accuracy of measuring volumes of gas in the colon*

A total of 9 gas volumes were introduced into the colon in the same two subjects.

To determine the accuracy of the method, a statistical analysis of the two colon gas experiments was undertaken as it was for the stomach gas experiments.

Figure 6 shows this plot from an experiment in which gas was introduced into the colon of subject R. M. The measured initial volume is 0 ml.

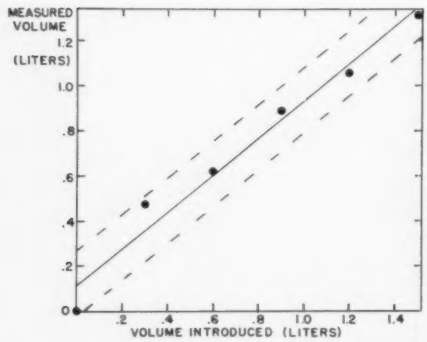


FIG. 6. GRAPH SHOWING THE RELATIONSHIP BETWEEN THE VOLUME OF GAS INTRODUCED INTO THE COLON AND THE INCREASE IN ABDOMINAL GAS VOLUME AS MEASURED BY THE PLETHYSMOGRAPHIC METHOD

Results of one experiment on subject R. M.

The Y intercept is 112 ml. The slope of the line of regression is 0.817. The standard error of estimate is 70 ml.

Figure 7 shows a similar plot from an experiment in which colon gas was introduced into subject A.B.D.B. The measured initial volume is 69 ml., the Y intercept is 106 ml. The slope of the line of regression is 0.90. The standard error of estimate is 60 ml. We conclude from this experiment that with straining, increments of pressure are equally transmitted throughout the abdominal cavity. Although the slope of the regres-

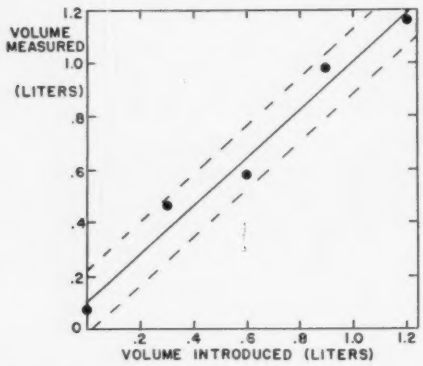


FIG. 7. GRAPH SHOWING THE RELATIONSHIP BETWEEN THE VOLUME OF GAS INTRODUCED INTO THE COLON AND THE INCREASE IN ABDOMINAL GAS VOLUME AS MEASURED BY THE PLETHYSMOGRAPHIC METHOD

Results of one experiment on subject A. B. D. B.

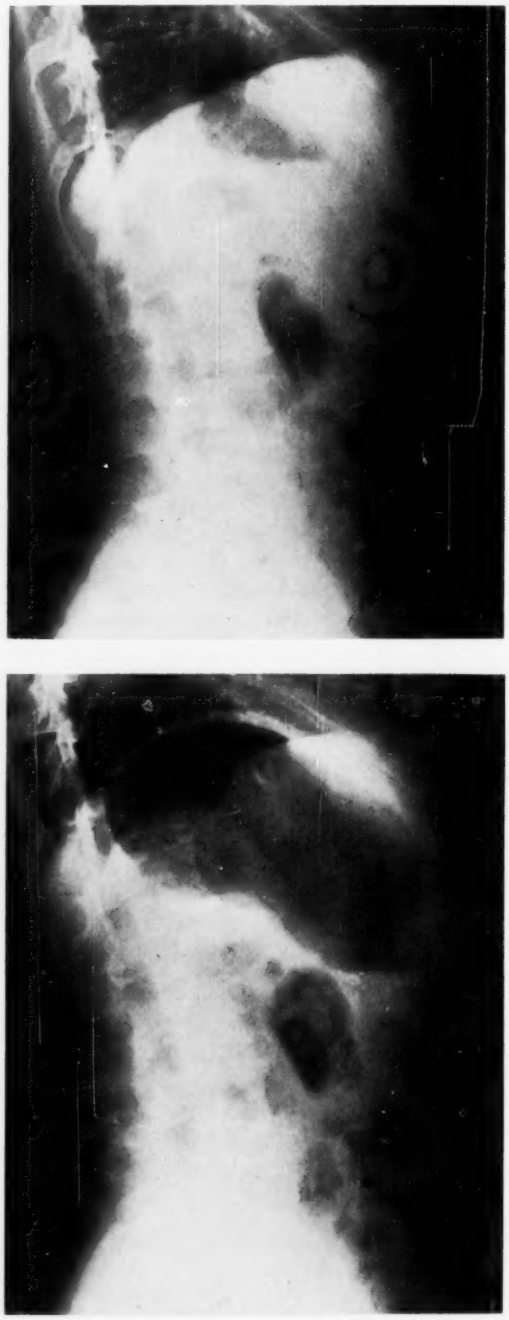


FIG. 8. RADIOGRAPHS OF THE ABDOMEN (a) CONTROL, (b) AFTER INJECTION OF 500 ML. OF AIR INTO THE STOMACH (SUBJECT R. M., 2/17/55)

TABLE I

Some effects of injecting gas into the colon

Subject	Total vol. of gas added to colon	Pressure in colon	Interval between last addition of gas and pressure measurement	Circumference of abdomen at umbilicus	Subjective feelings
	ml.	*cm. H_2O*		*cm.*	
R. M.	0	0	0 sec.	76	
	300	12	0 sec.	77	
	600	11	0 sec.	77	
	900	13	0 sec.	79	Slightly distended
		10	5 sec.		
		7–10	5 min.		
	1,200	11–12	0 sec.	79	Moderately distended
	1,500	13	0 sec.	82	Severely distended. Cramping pain
A. B. D. B.	0	0	0 sec.		
	300	17	0 sec.		
		12	2 min.		
	600	11	0 sec.		
		10	5 min.		
	900	9	0 sec.		Slightly distended
		8	5 min.		
	1,200	15	0 sec.		Severely distended. Cramping pain
		9	5 sec.		

sion lines should ideally be 1.00, the deviation of individual points from the regression line was sufficiently great so that they could conceivably have represented a line of slope 1.0. There is, however, doubt about the second decimal place.

The subjects noted that when the rectum became distended there would be pain, which was relieved when the gas bubbled up into the more proximal regions of the colon. A volume of 300 ml. introduced into the rectum brought the rectal pressure to about 10 cm. H_2O (Table I). Further introduction of gas caused transient rises in pressure which diminished to about 10 cm. H_2O after some of the gas escaped to other parts of the bowel. By the time that 1,200 ml. had been introduced, the pressure was still in the range of 10 cm. H_2O. At this point the subjects felt distended with gas and there was mild cramping pain. In R. M. a final volume of 1,500 ml. was reached at which point the rectal pressure was 13 cm. H_2O. It is apparent from the X-ray films (Figure 9) that the abdomen which contained 1,200 ml. of added gas was moderately to markedly distended by comparison with the control film.

3) *Volume of abdominal gas in normal subjects and in patients with pulmonary disease*

For this series of experiments, two groups of people were studied: (A) normal subjects and (B) patients with pulmonary disease. The studies were done at any time of the day, with no special preparation.

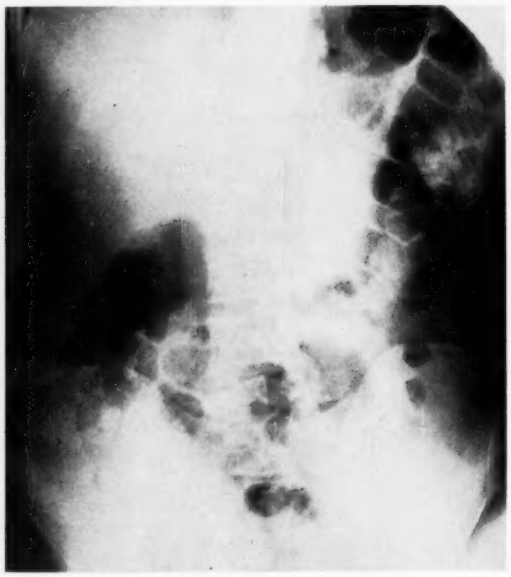
The patients with pulmonary disease were undergoing studies of compliance and airway resistance which necessitated placing a balloon in the esophagus. The balloon was passed first into the stomach, abdominal gas was measured, and then the balloon was withdrawn into the esophagus for the remainder of the studies.

(*A*) *Normal subjects.* A total of 32 observations is reported in 13 normal subjects, 11 men and two women; their age range was 20 to 43 years (Table II-A). There are multiple determinations on different days in several subjects. The average amount of abdominal gas in these subjects was 115 ml. (SD = ± 127 ml., SE = ± 23 ml.).

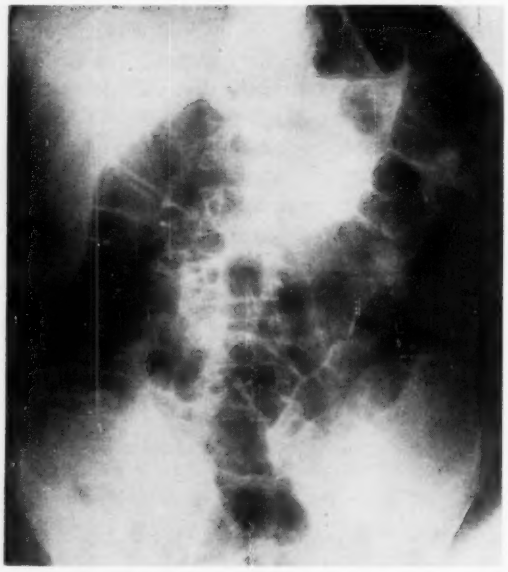
(*B*) *Patients with lung disease.* Forty-eight determinations of abdominal gas are reported from

47 patients with lung disease (Table II-B). One patient had two observations on different days. The patients consist of 35 men and 12 women and range in age between 15 and 76 years. The average amount of abdominal gas in these patients was 116 ml. (SD = ± 125 ml., SE = ± 18 ml.).

(a)



(b)



(c)

FIG. 9. RADIOGRAPHS OF THE ABDOMEN (a) CONTROL, (b) AFTER INJECTION OF 600 ML. OF AIR INTO THE COLON, (c) AFTER INJECTION OF 1,200 ML. OF AIR INTO THE COLON, COMPARE WITH FIGURE 6

TABLE II A

A) The volume of abdominal gas in normal subjects

Subject	Sex	Age	Date	Volume of abdominal gas	Subject	Sex	Age	Date	Volume of abdominal gas
				ml.					*ml.*
R. M.	M	32	11/12/54	34	A. B. D. B.	M	32	3/4/55	48
R. M.			12/22/54	63	A. B. D. B.			5/17/55	59
R. M.			1/13/55	182	G. B.	M	33	12/22/54	0
R. M.			1/19/55	67	G. B.			12/21/54	84
R. M.			1/28/55	261	G. B.			3/17/55	53
R. M.			2/3/55	84	R. S. M.	F	20	1/25/55	0
R. M.			2/4/55	132	R. S. M.			2/9/55	272
R. M.			2/7/55	106	E. L.	M	32		70
R. M.			2/10/55	55	M. R.	M	34		240
R. M.			2/17/55	158	P. C.	F	22		100
R. M.			3/31/55	0	J. M.	M	30		270
A. B. D. B.	M	32	11/12/54	110	E. R.	M	35		456
A. B. D. B.			11/18/54	0	D. G.	M	43		0
A. B. D. B.			12/7/54	0	E. P.	M	20		102
A. B. D. B.			1/17/55	0	E. H.	M	34		506
A. B. D. B.			1/18/55	0	K. S.	M	35		179

Mean volume of abdominal gas = 115 ml.
SD = ±127 ml.
SE = ±23 ml.

TABLE II B

B) The volume of abdominal gas in ambulatory patients

Subject	Sex	Age	Date	Volume of abdominal gas	Subject	Sex	Age	Volume of abdominal gas
				ml.				*ml.*
R. C.	M	30		233	E. P.	F	56	70
E. B.	F	17		375	O. H.	M	23	0
O. L.	M	51	1/14/55	134	J. K.	M	31	186
O. L.	M	51	2/3/55	0	C. E.	M	42	201
L. L.	F	32		0	H. W.	M	52	107
R. D.	M	45		92	J. D.	M	66	71
M. H.	F	52		118	J. H.	M	30	0
C. S.	F	46		13	R. M.	M	15	156
M. M.	F	52		103	G. D.	M	47	0
S. Z.	M	21		137	A. C.	M	76	108
W. E.	M	57		0	E. Z.	M	16	0
R. G.	F	45		56	W. M.	M	74	174
J. L.	M	65		108	J. D.	M	57	0
B. J.	M	28		0	J. W.	M	48	465
J. W.	M	45		104	C. P.	M	64	0
C. K.	F	47		0	H. B.	M	59	171
A. V.	M	56		200	A. S.	M	53	0
W. H.	M	54		128	A. C.	M	61	0
B. S.	F	30		0	R. C.	M	55	134
E. K.	M	63		537	L. H.	M	28	90
L. W.	F	49		210	I. M.	F	44	174
E. M.	F	49		0	J. W.	M	51	119
H. G.	M	54		244	R. N.	M	55	221
W. C.	M	36		330	R. W.	M	32	0

Mean volume of abdominal gas = 116 ml.
SD = 125 ml.
SE = 18 ml.

DISCUSSION

Although the method for measurement of abdominal gas is apparently simple in principle, it is not easy for the subject because of the special and unnatural type of effort required to compress gas in the abdomen without at the same time compressing the gas in the lungs. In this series of experiments, 7 out of 67 subjects were unable to per-

form the mechanical movements required by the test.

One of the theoretical questions in the method was whether change in gastric pressure is representative of change of pressure in the remainder of the gastro-intestinal tract. The finding that gas placed in the colon could be measured as accurately by this method as gas placed in the stomach seems to establish this point. In fact, colon gas seems to be measured more accurately than stomach gas as a comparison of the scatter diagrams will show. This is probably because large volumes of gas placed in the stomach are difficult for the subject to retain. For large volumes, failure to reach temperature equilibrium during compression would lead to underestimation of volumes. In a scatter diagram of stomach gas, 12 values are above the theoretical line and 24 are below it. Probably small volumes of gas escaped from the stomach during the experiments. Colonic gas was readily retained and there was no question of loss of gas by expulsion in these experiments. Since the tissues of the body are already equilibrated with 80 per cent nitrogen, the volume of air dissolved in gastro-intestinal fluids or absorbed through the mucosa must have been small during the 30-minute period of the test.

The Y intercepts of the regression lines calculated from measured volumes versus added volumes seem to be reasonably accurate, and this limits the possible random error in the initial measurement of abdominal gas.

The finding of an average volume of 0.1 liter is surprising in view of the previous report of one liter (1). The difference in results may be because of a difference in procedure. The present study was done at resting lung volume, whereas the prior measurements were made at the end of a full expiration. It can be shown that immediately after expiration, the expired gas condenses and cools in the box over a period of several seconds. Furthermore, at the end of a full expiration there may be a tendency for compression of alveolar air during further voluntary expiratory efforts. By radiographic estimation, the initial volume of gas is compatible with the plethysmographic finding of 0.1 liter, and is not compatible with an initial volume of the same order of magnitude as the volume added and subsequently visualized by X-ray. Addition of a liter of gas caused a subjective sensation of colic, or cramping abdominal pain, which was relieved by expulsion of the gas. Indeed the total volume added was limited by discomfort of the subject.

It can be deduced from the findings that the normal volume of gas in the intestinal tract is small and of the order of 0.1 liter. The presence of a volume of one liter in either stomach or colon is accompanied in normal subjects by discomfort and distension which are relieved by expulsion of the gas. This finding is of interest in relation to decompression because if the volume of gas normally present in the abdomen were 1 liter then explosive decompression, during which the abdominal gas expands two or three times, would be expected to produce considerable discomfort and pain in most subjects with the possible production of more serious damage to the bowel. When 234 subjects were explosively decompressed from 8,000 to 25,000 feet in 1 second, during which time the wet gas volume increases 2.2 times (4), 15 per cent had some abdominal distension and only 3 per cent had abdominal pain. These results are in keeping with a small normal abdominal gas volume such as has been found in this study.

The volume of gas in the lungs and abdomen is of importance in measuring the specific gravity of the body. A volume of 200 ml. of air in the body will give an error of .003 unit in measurement of specific gravity (5). The volume of gas that has been found in this study to be normally present in the abdomen is of the same order as the error of measurement of residual volume and so may be neglected, but if a larger volume of gas is present then its measurement is necessary in order to obtain an accurate value for specific gravity.

A method for the measurement of abdominal gas should also be of value for the quantitative assessment of the volume of gas in the gastro-intestinal tract in patients with a variety of gastro-intestinal disorders and in the evaluation of the action of carminatives and of gas absorbing agents.

SUMMARY

A physical method for determination of the volume of abdominal gas based on voluntary compres-

sion of the abdomen is described. This method is capable of measuring volumes of gas in the stomach within 180 ml. Gases in the colon are measured with equal accuracy.

The average volume of gas in the abdomen in 13 normal subjects was 115 ml. and the average volume of gas in the abdomen of 47 patients with lung disease was 116 ml.

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THE FAT, PROTEIN AND NUCLEIC ACID CONTENT OF THE LIVER IN MALNOURISHED HUMAN INFANTS

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In parts of the world where milk is in short supply babies after weaning are commonly put on a diet consisting mainly of carbohydrate. One result of this regimen is the development of a very fatty liver. The clinical and pathological features of these cases have been reviewed by Gillman and Gillman (1), Waterlow (2), and Trowell, Davies, and Dean (3). From several points of view it is of interest to know something about the composition of the fatty livers found in these cases. Earlier work suggested to one of us that the degree of fatty infiltration determined the severity of the disease, the response to treatment, and the likelihood of death (Waterlow, [2]). In the last few years, however, we have been able to measure the fat content of biopsy samples of the liver in a much larger series of cases. The results do not support the original conclusion, since no correlation has been found between the amount of fat in the liver and the clinical condition of the child (Waterlow [4]).

Microscopically, in most of the liver cells in these cases there is only a thin rim of cytoplasm surrounding a large fat globule. It seems possible that this may not be simply the result of distension of the cell by fat, as has usually been assumed; there may have been an actual loss of cytoplasm as well.

It is well known that when rats are starved or put on a protein-free diet, in a few days they may lose up to 40 per cent of their total liver protein, ribonucleic acid and phospholipid (Addis, Poo and Lew [5]; Kosterlitz [6]; Campbell and Kosterlitz [7]; Munro and Naismith [8]; Thomson, Heagy, Hutchison, and Davidson [9]). In none of these experiments, however, did the rats develop fatty livers. The fatty infiltration of the liver that is produced in rats by dietary means seems not to be accompanied by such large losses of liver protein. Presumably this is because the diets which most effectively produce fatty livers in rats, although deficient in lipotropic factors, are not necessarily very low in protein. In man, however, the case is different. The cause of the fatty liver in kwashiorkor is still unknown, but it is probably not simply a deficiency of lipotropic factors such as choline and methionine (Waterlow [2]). There is nearly always a history of a very low protein intake. That being so, it seems logical to suppose that there may be two processes going on side by side in these livers—accumulation of excess fat, and depletion of protein and other cytoplasmic constituents. The measurements described in this paper were undertaken to test this hypothesis.

Since in the living subject we cannot measure the weight of the liver and hence the total liver protein, it is necessary to relate all measurements to a fixed standard of reference. This problem of a standard has been discussed by Thomson, Heagy, Hutchison, and Davidson (9). They found, in agreement with the earlier work of Kosterlitz and Campbell (7), that in the adult rat deoxyribosenucleic acid (DNA) can be used as a standard of reference, since the total amount of DNA in the liver remained constant under a wide variety of dietary conditions. They also showed that under the conditions of their experiments there was no change in the average amount of DNA per cell. Thus changes in other constituents, when referred to DNA, could be interpreted as changes in the composition of the average liver cell.

Most of this experimental work was done on fully grown rats, and the conclusions cannot be applied without scrutiny to our cases, who were infants. The total DNA content of the liver is certainly not constant in the growing organism, nor does the average amount of DNA per nucleus remain unchanged. These points will be considered in more detail in the discussion. Although we recognize that DNA may not be a completely valid standard of reference, we have nevertheless

found it useful, as the best available approximation, for giving some insight into the changes occurring in the livers of our cases.

In this paper we report the results of measurements, before and after treatment, of the protein, DNA and ribosenucleic acid (RNA) content of the liver in a group of malnourished infants with fatty livers. Measurements of the activity of a number of liver enzymes have also been made at various times in the course of this work. Some of these results have already been reported (Waterlow [10, 11]; Waterlow and Patrick [12]).

METHODS

Preparation of tissues. Liver biopsy was done under local anesthesia (2 per cent w/v Procaine) by the transpleural route of Iversen and Roholm (13). The tissue was transferred to a piece of filter-paper moistened with saline, and put on ice in a stoppered tube. This procedure is open to criticism, since it may cause some alteration in water content. It was adopted in order to preserve the tissue as far as possible undamaged for enzyme assays. For measurement of water content by the method of Billing, Conlon, Hein, and Schiff (14) it is necessary to have a micro-balance by the bedside, which was not feasible for us. There was inevitably some delay before measurements could be begun, and the specimen usually remained on ice for about ½ an hour. Samples weighing 1 to 2 mg. were taken for enzyme assays. Another sample was fixed in Bouin's fluid for histological examination. A third fragment, weighing 1 to 2 mg., was weighed on a quartz fibre torsion balance, and then dried over phosphorus pentoxide. The water content was calculated by difference.

Fat was estimated by extraction of the dried tissue overnight with hot ethyl ether in a micro-Soxhlet apparatus. The tissue was dried and weighed, and the fat content calculated by difference. In unpublished experiments it was found that this simple method of extraction removes all the excess neutral fat but leaves behind lipid amounting to some 2 per cent of the fresh weight. This error, however, was not important, since we are dealing with livers containing a very great excess of neutral fat (Table II).

Nucleic acids were extracted from the dry fat-free tissue by the method of Schneider (15). The specimen was homogenized in ice-cold 5 per cent w/v trichloracetic acid (TCA), in a small glass homogenizer constructed as described by Steele, Sfortunato, and Ottolenghi (16). The homogenate was decanted into a tared tube, centrifuged, and the precipitate washed twice with cold 5 per cent TCA to remove acid-soluble nucleotides. The residue was then extracted with about 40 cu. mm. of 5 per cent TCA at 90° C. for 15 minutes. The exact volume of the extract was determined by weighing.

Total nucleic acids in the extract were measured spectrophotometrically by their absorption at 263 mμ. For this estimation 7 cu. mm. of extract were added to 170 cu. mm. of water. At this dilution the TCA blank has an optical density of only 0.02, whereas that of the samples was between 0.3 and 0.6. These measurements were made in a Unicam SP 500 spectrophotometer adapted for micro-cuvettes.

DNA was measured by the indole reaction of Ceriotti (17). Seven cu. mm. of extract were added to 170 cu. mm. of indole reagent. This reaction is extremely sensitive; measurements can be made with ease on extracts containing 0.1 μg. or less of DNA-phosphorus per aliquot of 7 cu. mm. All measurements were made in duplicate. In 50 consecutive estimations by the indole method the mean difference between duplicates was 4.7 per cent (S.E. ± 4.76). RNA was calculated by difference from these two measurements.

It was found that perchloric acid, which has been recommended by Ogur and Rosen (18) for the extraction of nucleic acids, decreased the intensity of the indole color, particularly if the extracts were left for any length of time in the refrigerator, whereas in TCA the nucleotides seem to be quite stable. It was for this reason that TCA was chosen as the extracting medium, although it has an appreciable absorption in the ultra-violet.

Calculation. In each set of indole measurements a standard was included made from semi-purified human DNA. This was prepared from post-mortem tissue by the method of Brues, Tracy, and Cohn (19), and had an optical density at 263 mμ. of 0.279 per μg. phosphorus per ml. (molar extinction coefficient = 8,950). This factor was used to calculate the contribution of the DNA, as measured by the indole reaction, to the ultra-violet absorption of the mixed nucleic acids. The total absorption at 263 mμ. minus the absorption due to DNA = absorption due to RNA. From this remainder the RNA concentration was calculated, using a factor derived from human RNA prepared by the same method (Brues, Tracy, and Cohn [19]). The RNA standard had an optical density at 263 mμ. of 0.303 per μg. phosphorus per ml. (molar extinction coefficient = 9,400). Results for both DNA and RNA are expressed in terms of nucleic acid phosphorus.

Measurement of RNA by difference has undoubted disadvantages, since errors tend to be magnified. The method was chosen, however, because it was found that the orcinol method of Mejbaum (20), as used by Schneider (15) and many subsequent workers, was insufficiently specific. Human liver contains large quantities of glycogen (see below), much of which is not removed by washing with cold TCA. The glucose that is produced on hydrolysis with hot TCA interferes in the orcinol reaction. The color produced by glucose has an absorption maximum at 540 mμ. (Drury [21]), whereas with ribose the maximum is at 660 to 670 mμ. However, even when corrections were made, based on readings at both wave-lengths, we were not able to get satisfactorily reproducible results.

Protein nitrogen was measured in the residue after extraction of the nucleic acids. The residue was washed into a thick-walled pyrex tube, 120 cu. mm. of 50 per

cent (v/v) H_2SO_4 were added containing 1 per cent (w/v) $CuSO_4$ and 1 per cent (w/v) SeO_2, and the tube was put in the oven at 100° until excess water had been evaporated off. The tubes were then heated in a brass block on an electric hot-plate at about 300° for at least 8 hours. After cooling, 1 ml. of water was added, and aliquots were Nesslerized by the method of Johnson (22) to give a final volume of 3.5 ml.

Glycogen was estimated in a few specimens by the method described by Patrick (23). This could not be done as a routine, because enough tissue was usually not available.

Sampling error. Although the pathological process in these cases—fatty infiltration—is relatively uniform, the liver is not an entirely homogeneous tissue. Therefore in such small fragments, which include only a few lobules, a large sampling error is to be expected. In preliminary studies on enzymes, duplicate estimations made on separate pieces of biopsy tissue agreed surprisingly well (Waterlow [10]). The variation in nucleic acid content seems to be larger. In two experiments six pieces of about 1 mg. fat-free dry weight were taken from specimens of liver obtained at necropsy. The results were as follows:

DNAP, μg. per mg. N.

Liver 1:	1.22,	1.16,	1.45,	1.00,	1.45,	1.30
Liver 2:	1.56,	1.61,	1.78,	1.45,	1.31,	1.81.

The variability is large, and it is to be expected that significant differences in nucleic acid content can only be established between groups of observations. A single measurement has little meaning when made on such a small amount of tissue.

TABLE I

Clinical features of cases investigated *

		Range
Number of cases	12	
Mean age, *months*	12.25	(6–19)
Mean weight deficit, *per cent*†	44±7.3	(35–54)
Mean liver enlargement, *cm.*‡	4±1.3	(2–7)
Number with edema: Severe	7	
Slight	3	
Number with dermatosis	4	
Number with mucosal lesions	5	
Number with dyspigmented hair	6	
Mean serum total protein, *g. per 100 ml.*		
On admission	4.4 ±0.84	(3.2–5.8)
On discharge§	6.85±0.35	(6.3–7.4)
Mean serum choline esterase, *Michel units*		
On admission	0.23±0.115	(0.08–0.50)
On discharge§	0.68±0.07	(0.61–0.82)
Mean weight gain on treatment, *kg. per week*	0.18±0.06	(0.09–0.32)

* Mean ± standard deviation.

† Deficit of weight below standard weight for age, as given by Holt and Howland (39). Weight measured after the loss of edema.

‡ Distance of liver edge below costal margin.

§ Excluding one case discharged before clinical recovery was complete.

CLINICAL DATA

The results reported here were obtained on 12 babies with fatty livers from whom more than one biopsy was available, taken before and after treatment. These were the only criteria for inclusion in the group. By a fatty liver is meant one in which fat forms more than 20 per cent of the fresh weight. Many observations were made on other miscellaneous cases, but they do not form a sufficiently homogeneous series for any conclusions to be drawn.

The general characteristics of this group are summarized in Table I. The picture is typical of kwashiorkor as it is seen in Jamaica (Waterlow [2]; Jelliffe, Bras, and Stuart [24]). All the babies were grossly underweight, yet most of them still retained some subcutaneous fat, often in considerable amounts; thus the reduction in tissue mass was probably even greater than appears from the figures for body weight. Clinically, a striking feature of these cases is the very severe loss of muscle substance. The buttocks, for instance, seem to consist of nothing but a fold of skin. Mucosal lesions and dermatosis, which are thought to be caused by vitamin deficiencies, were usually not severe. The reductions in serum protein and choline esterase are characteristic (Waterlow [2]; Dean and Schwartz [25]).

It is impossible to give an exact estimate of the diet before admission to hospital. The downhill course starts at the time of weaning, and in the average case the condition takes two or three months to develop. During this time the baby is fed mainly on starchy paps, of which the most popular is cornmeal porridge. The maximum milk intake was probably about ¼ pint of cow's milk per day, or its equivalent as condensed milk, giving a protein intake of 4 to 5 grams a day.

On admission to hospital the babies were put for the first few days on a diluted skim-milk mixture calculated to provide about 8 grams of protein a day. It had been found previously that an intake of this order seems just to maintain the *status quo*, and to produce neither improvement nor deterioration (Waterlow [2]). Frequently vomiting makes it impossible to secure a higher intake, and in severe cases it may be necessary to give transfusions of plasma or whole blood. Protein hydrolysates have not been used in this hospital. The treatment of these cases has been discussed in considerable detail in a recent publication (Waterlow [4]).

The initial biochemical and hematological measurements, including measurement of prothrombin time, were made during this preliminary period on a low protein intake. The first liver biopsy was done 3 to 7 days after admission to hospital, depending on the general condition of the child. After the biopsy the strength of the milk mixture was increased by stages until an intake of 2 to 3 pints of cow's milk a day had been achieved. Supplementary foods were then given. The average duration of stay in hospital of the cases in this series was 14 weeks. Progress during the first month or even two months was frequently very slow, but in the third month the rate of weight gain became very rapid. The average

TABLE II

Gross composition of the liver in malnourished infants *

Time of biopsy	Number of specimens	Per cent of fresh weight			Water, mg. per mg. fat-free dry weight	Protein,† % of fat-free dry weight
		Fat	Water	Non-fat solids		
(1)	(2)	(3)	(4)	(5)	(6)	(7)
On admission	12	33.8±6.5 (28.6–46.5)	51.7±5.9 (39.6–58.9)	14.3±2.15 (10.9–18.1)	3.71±0.73 (2.8–5.1)	50.0±13.1 (32–75)
After 3–8 weeks	11	8.3±5.6 (3.0–21.0)	69.0±6.5 (54.6–80.5)	22.7±3.3 (16.1–26.8)	3.17±0.71 (1.85–5.0)	45.2±8.6 (25–69)
After 9–24 weeks	7	3.1±2.0 (0.8–7.5)	73.7±1.7 (71.5–75.7)	23.4±1.6 (21.6–27.1)	3.36±0.55 (2.66–4.83)	52.1±14.0 (34–79)

* Mean ± S.D.
(Range).
† Protein calculated as N × 6.25.

rate of weight gain over the whole period of treatment was 0.18 kg. per week, which is about twice the normal rate for this age. Whenever possible two more biopsies were done, one during the recovery period and one just before discharge.

Ideally in a study of this kind it would be desirable to have control material from normal babies. In practice, however, this was not possible. The vast majority of our cases come from the lower economic strata: in the post-weaning period, few, if any babies of this group could be considered absolutely normal by European or American standards. Even if such babies existed in our hospital population, the pressure on bed space and the fact that liver biopsy is not a procedure entirely devoid of risk, would make it unjustifiable to admit them for investigation as a control group. On the other hand the malnourished infants described in this report were retained in hospital for as long as possible, and at the end of treatment represent the best approximation to the "normal" that we can achieve.

The same considerations apply to the cytology of the liver. The livers at the end of treatment show neither fatty infiltration nor fibrosis. They do, however, present two phenomena which might be considered abnormal: a tendency to slight cellular infiltration of the portal tracts, and an apparent increase in the number of large nuclei and binucleate cells—in other words, an increase in the degree of polyploidy. Both these phenomena have been repeatedly mentioned by workers in the tropics (Walters and Waterlow [26]; Gillman and Gillman [1]). The significance of the apparent increase in polyploidy is being investigated.

RESULTS

In human beings it is seldom that tissue is available for analysis at the instant of death. In the interval that elapses before necropsy significant chemical changes may occur. It may therefore be of interest to record some figures for the gross composition of liver specimens obtained by biopsy. Even these give only an approximate picture, since some glycogen was undoubtedly lost while the tissue was standing on ice. The results are shown in Table II.

The water content per unit fresh weight is naturally very low in the fatty livers. When related to fat-free dry weight (column 6) the water content is slightly but not significantly higher in the fatty livers than in the specimens taken after treatment. These figures, however, cannot be considered very accurate because the tissue was rinsed in saline after being removed from the biopsy needle. Subsequent measurements on both post-mortem and biopsy tissues suggest that there is a real increase in water content in the livers of these malnourished cases (to be published).

As might be expected, the protein, expressed as a percentage of the fat-free dry weight (column 7) shows no change as a result of treatment. The variability of these figures is high, probably because of varying amounts of glycogen. It was surprising to find that protein accounted for only about 50 per cent of the dry weight after ether extraction, and the question therefore arose: what is the nature of the residue? Total nucleic acids (DNA plus RNA) amount to 2 to 3 per cent. The fat extraction by ether is incomplete; some lipid is left behind, amounting to about 2 per cent of the fresh weight, or 10 per cent of the dry weight. Measurement of the ash content in two cases gave figures of 5.3 and 3.6 per cent. Finally, in 10 livers glycogen was estimated in the dried tissue; the average amount found was 21.7 per cent

(range 10.7 to 38.2 per cent) of the fat-free dry weight. This is certainly an under-estimate of the true amount present *in vivo*. In a few cases samples of liver were put directly into 30 per cent (w/v) KOH at the bedside immediately after biopsy; duplicate samples were stood on ice in the usual way. The results showed that one-third or more of the glycogen may be lost during the half-hour or so that the tissue stood on ice before being weighed and dried. If it is assumed that the true glycogen content is of the order of 30 per cent of the fat-free dry weight, then almost all the undetermined part of the residue is accounted for, and analysis of the ether extracted material would show the following composition, in round figures:

	%
Protein	50
Lipid	10
Nucleic acids	2
Ash	4
Glycogen	30
Undetermined	4
	100

We had not expected to find such large amounts of glycogen, since the babies were fasted overnight before biopsy. There does not seem to be any consistent difference between the glycogen content of livers from malnourished and from treated cases. The severe fatty infiltration evidently has not impaired the liver's capacity to form and to store glycogen. This confirms the histological finding, that in sections stained by the periodic acid-Schiff method large amounts of glycogen are present even in cells which are grossly distended by fat globules. Very different is the picture seen in another group of Jamaican children, suffering from the disease locally called "vomiting sickness," which seems to be a toxic condition. In these children the liver is almost totally depleted of glycogen, both chemically and histologically, and the blood-sugar is reduced almost to zero (Jelliffe and Stuart [27]; Patrick [23]).

In Table III are shown figures for the protein-nitrogen, DNA and RNA content of the liver. The cases are the same as in Tables I and II. It is evident that at the time of the first biopsy—that is, before treatment—there was a severe depletion of liver protein, as judged by the low ratio of protein-N to DNA–P. The difference in N/DNA–P between the first and second biopsies is statistically significant ($P = 0.01$). That between the second and third biopsies is not significant. If the first and third biopsies are compared, it seems that these infants, at the time of their admission to hospital, had lost on the average some 40 per cent of their liver protein. A loss of the same order was found by Kosterlitz (6), by Munro and Naismith (8), and by Thomson, Heagy, Hutchison, and Davidson (9) in the livers of protein-starved rats.

The difference in RNA–P/DNA–P between the first and second biopsies is significant at the level of 5 per cent. The last column of Table III suggests that either the initial loss of RNA is less severe than the loss of protein, or that RNA is resynthesized more slowly.

It might be expected that the greater the degree of protein starvation the lower would be the N/DNA–P ratio, and that there might therefore be some correlation between this ratio and the general condition of the baby. This expectation has not been fulfilled, partly, no doubt, because of sampling and analytical error, but partly for other reasons which will be discussed below.

Cases that die may reasonably be regarded as the most severe unless there is some complicating factor. In six fatal cases liver tissue was obtained

TABLE III

Protein, DNA and RNA content of the liver *

Time of biopsy	Number of specimens	μg. per mg. fat-free dry wt.			Protein N / DNAP, μg./μg.	RNAP / DNAP, μg./μg.	RNAP / N, μg./mg.
		Protein N	DNAP	RNAP			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
On admission	12	80±20.6	1.73±0.53	1.96±0.93†	49±15.9	1.15±0.41	0.25±0.11
After 3–8 weeks	11	71±12.7	1.15±0.39	1.59±0.45	69±16.7	1.53±0.39	0.22±0.06
After 9–24 weeks	8	83±25.4	1.12±0.30	1.40±0.14	83±28.8	1.37±0.29	0.17±0.06

* Figures are mean ± standard deviation.
† If one very high figure (RNAP = 4.60 μg. per mg.) is omitted, this becomes 1.71 ± 0.53.

by the usual puncture technique within 30 minutes of death. In one other baby included in this group a biopsy was done one day before death. It had been expected that in these fatal cases both N/DNA–P and RNA–P/DNA–P would be low, but this was not found. The results in these seven cases were as follows:

N/DNA-P: (μg./μg.)	mean 63.0	range 43–95
RNA-P/DNA-P: (μg./μg.)	mean 1.86	range 1.13–2.44

It was particularly unexpected to find that RNA–P/DNA–P was on the average higher even than in the livers of treated cases. Some specimens taken at autopsy one or more days after death have shown similarly high figures.

These results could be explained if there had been a loss of DNA from the liver. According to Leuchtenberger, Leuchtenberger, and Davis (28), post-mortem change should not produce significant effects in such a short time. It is possible that some destruction of DNA may have occurred before death, such as is known to occur when there is pyknosis of the nucleus (Leuchtenberger [29]). This, however, is only a suggestion for which we have no evidence. The microscope does not give us much information on this point, because the nucleus tends to be distorted by the accumulation of fat in the cell. This point is being investigated.

DISCUSSION

The validity of using DNA as a standard of reference for other measurements has been discussed in detail by Davidson and his colleagues (Thomson, Heagy, Hutchison, and Davidson [9]). They confirmed and extended the earlier observations of Campbell and Kosterlitz (7), that in the adult rat the amount of DNA per cell and per liver is constant over a wide range of dietary conditions. In the growing animal the position is less clear. In man during growth there is an increase in the proportion of large, presumably polyploid nuclei (Böhm [30]). In the rat several authors have found an increase in the average amount of DNA per nucleus (Thomson, Heagy, Hutchison, and Davidson [9]; Fukuda and Sibatani [31]; Jacob, Mandel, and Mandel [32]).

During growth there is also a great increase in the total number of liver cells. The joint effect of these two processes—multiplication of cells and increase in DNA per cell—is that the total liver DNA rises roughly *pari passu* with body weight (Campbell and Kosterlitz [7]; Jacob, Mandel, and Mandel [32]). Of these two processes the former is quantitatively far more important. The data of Jacob, Mandel, and Mandel (32) show that in rats between the ages of 8 and 60 days the total liver DNA increased 12 times; the average amount of DNA per nucleus increased only by a factor of 1.3, so that there must have been a 9-fold increase in the number of cells.

Little is known about the effect of malnutrition on these two processes. Ely and Ross (33) found an increased DNA content per nucleus, compared with controls, in young rats kept for periods of up to 7 weeks on a protein-free diet. Fukuda and Sibatani (31) showed that when young rats were put on a restricted diet so that they no longer gained weight, the amount of DNA per liver cell nucleus still continued to increase in the normal way. Thus the DNA per cell seemed to depend on the age or the ideal weight of the rat, and not on its actual body weight.

In neither of these papers are figures given for liver weights, from which it would be possible to calculate the total liver DNA or to estimate the number of cells. Histological observations suggest that malnutrition slows down the multiplication of liver cells. Balfour (34) found that when young rats are given an adequate diet after a period on a ration low in protein (cassava) there is a burst of mitotic activity in the liver. This fits in with the suggestion of Thomson, Heagy, Hutchison, and Davidson (9), in discussing the work of Ely and Ross (33), that protein deficiency might inhibit growth and mitosis to a greater extent than it affects the premitotic synthesis of DNA.

It is therefore difficult to assess from the experimental work the probable effect on liver DNA in our human cases of a period of malnutrition followed by a period of treatment with a good diet. It seems reasonable to suppose that during treatment the amount of DNA per cell either remained the same or increased slightly. Histologically, in biopsies taken after one month or more of treatment, large, probably polyploid, nuclei seemed to be more numerous than in the initial biopsies. This suggests that there may well have been some

increase in the average DNA content per nucleus, which would tend to mask simultaneous increases in protein and RNA. It would be desirable to check this point, either by spectrophotometric measurements by the method of Pollister (35), or by making nuclear counts, and relating them to the total DNA content of the specimen. Work on these lines is proceeding. In any case, however, an increase in DNA content per nucleus would not invalidate the conclusions that have been drawn. On the contrary, the differences that have been found between the first and subsequent biopsies in the ratios of RNA and protein to DNA would become all the more significant.

It is also probable that during treatment there was an increase in the total number of liver cells. No excess of mitoses was observed, but the increase in number of polyploid nuclei is usually regarded as evidence of regeneration and multiplication of cells. It is therefore likely that the increase in total liver protein was even greater than is shown by the figures for protein/DNA.

A further complication is the fact that the liver is by no means a homogeneous tissue. A few cell-counts that were done on infant livers in Africa (Walters and Waterlow [26]) showed that in the most normal livers available only two-thirds of the cells were hepatic cells. From a comparative point of view it would not affect the results if the proportion of hepatic and non-hepatic cells were constant. No actual counts have been made in the livers of this series, but histological examination gives the impression that there is a slight cellular infiltration in the specimens taken after treatment. These infiltrating cells probably contain less cytoplasmic protein and RNA per unit DNA than the hepatic cells proper; therefore an error introduced in this way would work in the same direction as the error caused by an increase in DNA content per nucleus, and the differences that have been found between specimens before and after treatment would again tend to be under-estimates rather than over-estimates.

There is a third factor which influences the interpretation of these results. When an animal is put on a protein-free diet its liver loses a large proportion of its protein, but this loss does not increase progressively with time. For instance, Campbell and Kosterlitz (36) found that the reduction in liver nitrogen on a protein-free diet was no greater after 7 days than after 4 days. This is in keeping with the fact that the liver has a much more rapid turnover of protein than the so-called carcass (Sprinson and Rittenberg [37]). Muscle loses its protein more slowly than the liver (Addis, Poo, and Lew [38]), and presumably goes on doing so for a longer time. Thus the liver of a child which has been on a protein-deficient diet for 3 months will not necessarily be more depleted than the liver of one who has been on the same diet for one month.

It had originally been our hope that these measurements made on the liver might provide some index of the severity of protein depletion. Although they are not suitable for routine use, they might still provide a baseline for other and simpler measurements. The results suggest that useful statistical information can be obtained about a group of cases, but that a single measurement is of little value as a guide to the severity of an individual case. Even with an increase in the precision of the methods, it seems unlikely that this objective can be achieved, because of the sampling error and of the other sources of variability that have been discussed. Nevertheless, it is possible that an extension of these measurements to a wider variety of cases, and to other tissues, such as muscle, might give further insight into the type and degree of protein depletion produced by various forms of dietary deficiency.

SUMMARY

1. Measurements made on biopsy specimens of the liver from a selected group of severely malnourished infants show a great excess of fat, a slight and doubtful increase in water content, and a high concentration of glycogen.

2. The protein and ribosenucleic acid (RNA) contents of the biopsy specimens were measured and related to deoxyribosenucleic acid (DNA). These measurements were made soon after admission to hospital, and repeated after one to two months on a high protein diet.

3. On the assumption that the amount of DNA per cell and per liver remains constant, the results show that at the time of admission these cases had on the average lost some 40 per cent of their liver protein and RNA.

4. Evidence is discussed suggesting that the assumption of a constant DNA content is not

valid in this material. However, the errors introduced by this assumption tend to decrease, rather than to exaggerate, the differences found between biopsies before and after treatment.

5. It is concluded that measurements of this type may give useful statistical information about a group of cases, but they are not precise enough to serve as an index of the degree of protein depletion in a single case at a single moment of time.

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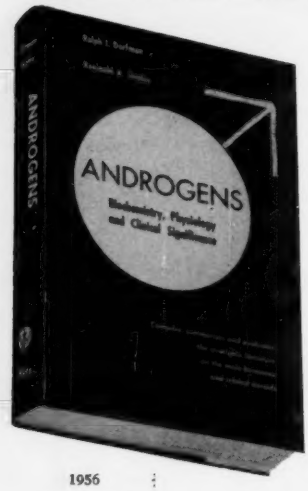

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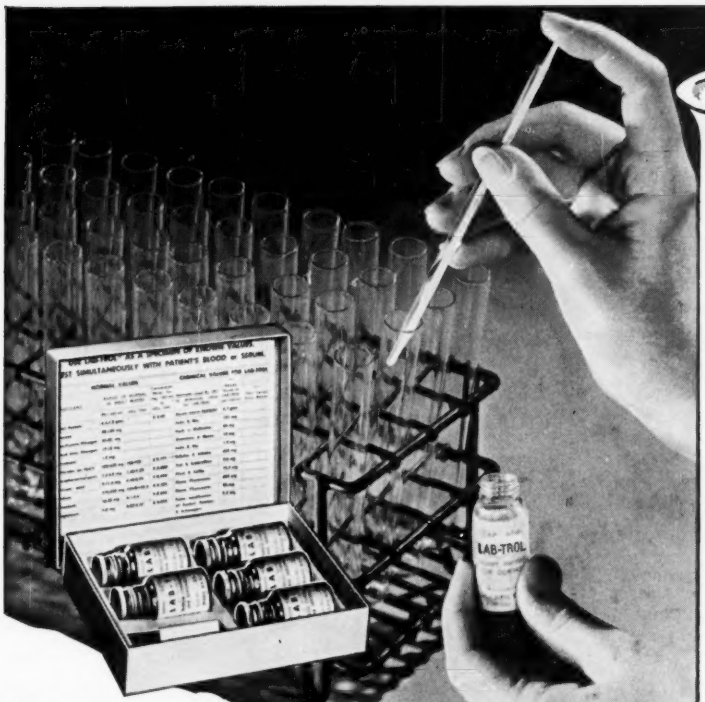

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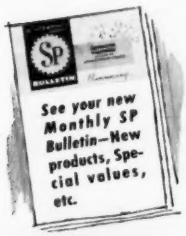

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